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ETIOLOGY

OF EPIZOOTIC ENCEPHALITIS OF THE RABBIT,

IN ITS RELATIONSHIPS WITH THE EXPERIMENTAL STUDY

OF LETHARGIC ENCEPHALITIS

ENCEPHALITOZOAN CUNICULI (nov. spec.)

by C. LEVADITI, S. NICOLAU and Miss R. SCHOEN.
(Pasteur Institute.)

(With plates III and IV.)

CHAPTER I

HISTORIC GENERALITIES

The etiological problem of lethargic encephalitis seemed definitely resolved, thanks to the experimental researches of Strauss, Hirshfeld and Loewe, Mc Intosh and Turnbull, Levaditi, Harvier and Nicolau, Doerr and Schnabel, Berger, etc. (1), when, in 1922, Kling and his collaborators, Davide and Liljenquist (2), brought to light facts which, at first glance, seemed to undermine the earlier formulated conclusions. One knows that, according to Levaditi, Harvier and Nicolau, confirmed by Doerr, Schnabel and Berger, lethargic encephalitis, transmissible to the rabbit, to the guinea pig, to the mouse, and, sometimes, to catarrhinian monkeys, is due to a filterable virus, which, inoculated by cerebral method, kills the

(1) Cf. for the literature, C. LEVADITI, Ectodermoses neurotropes, 1922, Paris, Masson.

(2) KLING, DAVIDE AND LILJENQUIST. The works of these authors, published, for the most part, in the C. R. of the Biology Society are found united in the Communications of the Bacteriological Laboratory of the Swedish State, 7, 1923.

animal in five to eight days, with clinical signs and microscopic alterations of acute encephalitis. Further more, this virus, deposited on the scarified cornea of the rabbit, provokes a kerato-conjonctivitis, source sooner or later of manifestations of mortal nevraxitis (Levaditi and Harvier). Researches of crossed immunity, incited by the comparison between encephalitis and experimental herpes (Blanc (1), and realized by Doerr and Schnabel, as well as by Levaditi, Harvier and Nicolau, had, in addition, demonstrated that the encephalic virus belongs to the same group as the herpetic germ, of which it is only, in the last analysis, a variety with eminently acute neurotropic affinities.

Now, Kling and his collaborators, on the occasion of a severe encephalic epidemy in Lapon (Sweden), undertook experiments in order to verify the statements of Levaditi, Doerr and Schnabel, etc... These experiments first of all assumed a confirmative nature. But, afterwards, the problem changed its nature. The Swedish authors succeeded in confering the encephalitis to the rabbit, in inocculating materials of human encephalitis (neuralgia, liquid cerebrospinal, filtrated fecal materials), proceeding from mortal cases or not. Nevertheless, they themselves perceived very quickly that the experimental sickness differed notably from that studied by Levaditi, Harvier and Nicolau, Doerr and Schnabel, Berger, etc... Although the rabbits inoculated with the herpetic-encephalic virus succumbed in the few days which followed the inoculation, the animals infected with the "Swedish virus" died later on, after a few weeks, sometimes after many months; most often, there was neither death nor sickness, so to speak. The success of the experiment in these cases was only proven by the anatomical-pathological lesions which presented the nevraxe of rabbits sacrificed long after the inoculation. Besides, these lesions offered an aspect entirely different from the alterations provoked by the herpetic-encephalic germ. It was a question of, not the meningeal and parenchymatous modifications of a clearly acute nature, which is constant in true encephalitis, but of chronic lesions (meningitis with mononuclears, peri-vascular disks, and, principally, nodules with epithelioid and gigantic cells; for details, see page 661.

The "Swedish virus" produced no keratosis followed by encephalitis. Although appearing capable of traversing the filter candles, as the herpetic-encephalic germ, this virus offered several particularities allowing one to distinguish it from the other. The action of heat, in particular, showed that Kling's virus resisted temperatures that totally annihilated the pathogenic activity of the filtrable encephalic and herpes microorganism.

Another difference, no less striking, resulted from the frequence, truly extreme, of successes which the experimental tentatives of Kling and his collaborators conveyed. Whereas elsewhere the positive results were exceptional, despite a great number of inoculations, practiced with the most diverse materials, gathered up on the living body as well as on the

(1) BLANC. C. R. of the Academy of Sciences, 182, 1921, p. 725.

cadaver, the Swedish authors saw their efforts come to a head, so to speak, each time that they attempted the experiment. Instead of four to five rootstocks of herpetico-encephalic virus, isolated with great pains by their predecessors, Kling and his collaborators obtained from them a far more considerable number, with infinitely less effort.

It became evident that the hypothesis after which the "Swedish virus" was only an attenuated variety of the herpetic-encephalic germ, hypothesis formulated, in the beginning, by Levaditi and Nicolau, was no longer supportable. Too many facts, better observations, ground it into a hole. The preceeding established data had thus to be interpreted from an entirely different manner: this is what Kling and his collaborators did.

For the other Swedes, all those who pretended to have in their hands the etiological agent of epidemic encephalitis were victims of a grave error. They had isolated the herpetic virus, whereas they believed to cultivate on the animal the germ of the v. Economo sickness. In effect, herpes complicates a multitude of infectious processes; why would it not add itself, in name of secondary sickness, to the lethargic encephalitis? In fact, had not Levaditi and Harvier called attention to, in the sick Hof ..., from which came their rootstock C, the presence of a facial herpes? According to all probability, affirmed the Swedish authors, Levaditi and Harvier, as well as Mc Intosh and Turnbull, Doerr, Schnabel, Berger, etc..., isolated, not the etiological agent of encephalitis, but very simply the herpetic virus, which had invaded the nevraxe by means of lesions provoked by the authentic germ of the v. Economo malady (cf. Kling and his collaborators (1)).

This authentic germ is the "Swedish virus". It alone must be considered as being the causal agent of epidemic encephalitis. This conclusion, formulated by Kling and his collaborators, thus implanted a completely new aspect to the etiological problem of the v. Economo sickness. Was it justified? The future is charged with demonstrating the contrary, as we will prove in the course of this Memoir.

We will leave aside, for the moment, the question of the etiology of epidemic encephalitis, in relation with the herpetic-encephalic virus. We will expose the actual state of the problem in a conference with "Medical days of Brussels", next June, in insisting on the arguments that authorize us to consider this virus as being the etiological agent of the v. Economo sickness(2). We will limit ourselves to the exposition of data which conducted us, little by little, to put in doubt Kling's and his collaborators' conception and, finally, to identify the experimental encephalite studied by this scientist with a spontaneous and epizootic infection of

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(1) KLING, DAVIDE AND LILJENQUIST. C. R. of the Society of Biology, 40, 1924, p. 514.

(2) This conference took place in the course of the first "Medical Day", June 29; it will soon be published.

the rabbit, whose clinical and anatomic-pathological particularities had been precised by several American and English authors, and whose microbe had been recently discovered.

Mr. Kling having had the kindness of entrusting us with his "Swedish virus" in passage, we began by confirming his assertions on the subject of the principle characteristics of this virus. Here is what we ascertained in the course of our experiments:

November 16, 1922, quite a large number of rabbits had been inoculated by cerebral method, with the Swedish virus of passage, conserved in diluted glycerin. Here are the results obtained:

A. In a first serie, we used young rabbits (one month of age). Rabbit 99/V and 98/V died the 29th day; rabbit 97/V succumbed the 37th day; rabbits 96/V and 98/V were sacrificed the 61st day. No lesion of the nervous system in these animals.

B. In a second series, we used adult rabbits:

RABBITS

	LAPINS	SACRIFIÉ LE : — DAY	LÉSIONS	PASSAGE	SACRIFIÉ LE :	LÉSIONS
1°	92/V	8° jour	0	lapin 70/M	93° jour	0
2°	84/V	15° jour	0	lapin 23 ; lapin 30	113° jour	0
3°	85/V	20° jour	0	lapin 7/A ; lapin 52	168° jour	0
4°	86/V	76° jour	0	"	"	"
5°	87/V	76° jour	+	lapin 57/E	mort le 118° jour DEAD	+++
6°	89/V	105° jour	++	"	"	"
7°	91/V	131° jour	++	"	"	"
8°	94/V	131° jour	++	"	"	"
9°	88/V	131° jour	++	"	"	"

Passages were effectuated in inoculating an encephalic emulsion by corneous and intracerebral methods. Not one of these animals presented keratosis. A single one, among these rabbits, was noticeably sick: it was rabbit 57/E, who, one hundred seventeen days after the inoculation, presented signs of leziness and weakness; it died the next day.

These experiments showed that, in accordance with Kling's affirmations, the "Swedish virus" is pathogenic for the rabbit. The infection that it provokes is not manifested, in general, by any apparent clinical sign (a single one of our animals was clearly sick before succumbing). It only manifests itself by microscopic alterations(see further), which appear after a very long incubation period (seventy-six, one hundred five, one-

hundred thirty-one and one hundred eighteen days). It is interesting to notice that the passages, made with encephals exempt of microscopic lesions (animals sacrificed during the incubation period), rested negative, whereas an injection practiced with the lesioned brain of rabbit 87/V, gave a clearly positive result. It would thus seem that the virus would appear in the nevraxe at the same time as the histological modifications that it provokes.

Later on, our tentatives of passage from brain to brain stayed unfruitful. The series found themselves interrupted, despite our insistance to continue them regularly, and that, whatever was the mode of inoculation and the method of penetration. We know actually that these failures were due to the fact that we would administer to our animals virus which was conserved too long in pure sterilized glycerin. In effect, the Swedish germ has this in particular that, contrary to the true filterable virus (poliomyelitis, rabies, encephalitis, herpes, etc.), it loses quite rapidly its virulence by conservation of brain fragments in the concentrated glycerin, at the temperature of the refrigerator. Nothing surprising since, just as we will see in the course of this Memoir, the Swedish germ is not at all a filterable virus, but a Microsporidia, a more fragile protozoan than the Ultra-microbes of Ectodermoses neurotropes.

Another statement struck us from the beginning of our research. Mr. Kling having made us get fragments of human encephalic brains, plunged in diluted glycerin, we inoculated these fragments in numerous rabbits by cerebral method. Not one of these animals contracted acute encephalitis; not one of them presented any more lesions of the nevraxe, at the time of a later examination practiced several months after the inoculation. Now, the same materials, injected by the scientists of Stockholm, into rabbits originating from Swedish rearing, had determined chronic encephalitis of which it was a question above.

There was in this something impressive that it was necessary at all cost to elucidate. Results so different could not depend on technical divergences, the two laboratories utilising the same procedures of inoculation. Only differences between the varieties of animals (rabbits) employed in Sweden and Paris could explain the observed deviations. That was the reason which made us determined to ask Mr. Kling to send us a lot of rabbits belonging to his rearing, so that we could simultaneously experiment on them and on rabbits of the Parisian region. He did this very amicably in July 1923. Now, it is these attempts which would end in results permitting us to resolve the problem of the nature of the Swedish encephalic virus.

In between time, we had knowledge of a complete series of works concerning a spontaneous epizootic malady of the rabbit, characterized by chronic encephalic alterations and reported in the United States and in England. The idea came to us that, seemingly, this malady, rare in the Parisian region, since, until then, in spite of the examination of several thousands of encephalic viruses, we had never happened upon it, had to be frequent in the Northern countries, and particularly in Sweden. If this hypothesis

found itself confirmed, one could suppose that chronic encephalitis observed by Kling and his collaborators, following inoculations of human materials, was none other than spontaneous encephalitis of the rabbit. This one would appear above all in animals whose brain was traumatized by experimental inoculations, such as they may be. The germ of this malady, living in the latent state in some organ (principally in young rabbits), would localize itself on the nevraxe and would produce chronic lesions each time that one would inject cerebral emulsions or others into the encephal, by themselves non-virulent. The results, so constantly positive registered by Kling and his collaborators, would explain themselves, in this case, by the intervention of a secondary spontaneous infection.

But this was only pure hypothesis, an hypothesis envisaged besides by Mr. Kling himself, who had soon isolated it on the faith of microscopic examinations of encephalic viruses of non inoculated rabbits, examinations which had ended in totally negative results. There was thus place to verify it with all the rigor that this type of research involves. This is what we did without delay.

Before undertaking the exposition of our results, it seems useful to us to review the information that was possessed (June, 1923 on the clinical and anatomical-pathological characteristics of epizootic encephalitis of the rabbit.

EPIZOOTIC ENCEPHALITIS OF THE RABBIT. — In 1917, Bull (1), examining histological encephalitis of rabbits infected with streptococci, there discovered microscopic alterations of encephalitis, whose particularities he describes (perivascular and nodular disks); the same lesions existed in three rabbits who died of septicemia, and who had never been inoculated. Later (1922), Oliver (1) (San Francisco) observed analogous alterations in rabbits injected with variable doses of asphenamine. In a first series of experiments, the animals were intoxicated by growing quantities of asphenamine; they died around the tenth day with microscopic signs of encephalitis. In a second series of attempts, it was a question of animals who succumbed as soon as the injection was medically administered, and nevertheless they also presented alterations of the nevraxe. In these conditions, it was impossible to admit a relationship of cause and effect between the administration of the medicament and the genesis of the cerebral-medullar lesions. The force of circumstance was thus to conclude that it was a question of a spontaneous infection, not manifesting itself by any appreciable clinical symptom. In reality, around 20 p. 100 of rabbits examined in the Oliver Laboratory were contaminated. The author minutely describes the microscopic alterations of the nervous system, which consist in meningitis with mononucleers and plasmatic cells, in peri-vascular disks

(1) OLIVER. The Journal of Infectious Diseases, 30, 1922, p. 91.
(2) C. C. TWORT. The Veterinary Journal, 78, no. 6, 1922.

recembling disks that one encounters in human or experimental encephalitis, and in nodules. These last ones are constituted by a mononuclear infiltration of the cerebral parenchyma; their center is necrosed and filled with drops of fat. Not one microorganism was able to be found by Oliver, despite the variety of the utilized bistological methods.

The same epizootic disease of the rabbit was affirmed, in England, by C. C. Twort (2). This author inoculates rabbits with lymphadenome; these show cerebral alterations, giving evidence of a state of chronic encephalitis. Nevertheless, examination of the control animals, originating from the same rearing, ends in the same results. It is thus a question of a spontaneous infection having no relationship with lymphadenome. This infection terminated itself sometimes by death, happening after a period of weakening and of convulsions. Twort brings attention to hypothermie, muscular debility, modifications of liquid cerebrospinal (lymphocytosis), and describes the microscopic alterations already studied before by Oliver and Bull. The existence of the malady in the endemic state, in certain rearings, renders difficult the study of its experimental transmission (by contact or by inoculation). Nevertheless, certain tentatives of infection by cerebral or peritoneal method realized by Twort, seemed crowned with success.

The name spontaneous encephalic-myelitis of the rabbit is relatively IMPROPER TO DESIGNATE THE INFECTION STUDIED BY Bull, Oliver and Twort. In effect, the central nervous system is not the only one to act up: the liver, the spleen and above all the kidney are the seats of lesions, whose characteristics were precised by Bell and Hurtzwell (1), and above all by C. C. Twort and H. E. Archer (2). The first ones confirm an interstitial lymphoctic nephritis, with nodules and atrophy of excretory tubes, in about 15 p. 100 of the rabbits examined (400 in total). Twort and Archer observe, on their part, kidney alterations, accompagnied by "splenic artery" and "hepatitis", in rabbits stricken by spontaneous encephalitis. These alterations consist in lymphocytic centers, giving place to an inflammatory and degenerative nephritis, whose intensity contrasts with that of the cerebral lesions. Actually, according to Twort and Archer, the kidney can be hardly touched [cf., on the subject of urinary and sanguine modifications observed in the course of spontaneous nephritis, the work of C. C. Twort and Archer (3)].

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(1) BELL and HURTZWELL. Journ. of Infect. Diseases, June 1919, p. 628 (cited according to Twort).

(2) TWORT and ARCHER. The Lancet, 1, 1923, p. 1102.

(3) The spontaneous malady of the rabbit was studied also by BONFIGLIO (Boll. e Atti delle Reale Academia medica di Roma, 50, 1923-24). The author seems to ignore the prior works of Bull, Oliver and Twort.

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There are the principle clinical and anatomic-pathological characteristics of the epizootic encephalitis of the rabbit. As we have said, the perusal of the works of Bull, Oliver, Twort and Archer, suggested to us the idea that, seemingly, between encephalitis provoked by the "Swedish virus" of Kling and this spontaneous nevrasitis, there had to be close relationships, if not absolute identity. We asked our old collaborator C. C. Twort to kindly entrust to us some materials of epizootic encephalitis, in order to compare the lesions of this infection to those of the malady studied by Kling, Davide and Liljenquist. This is what he did.

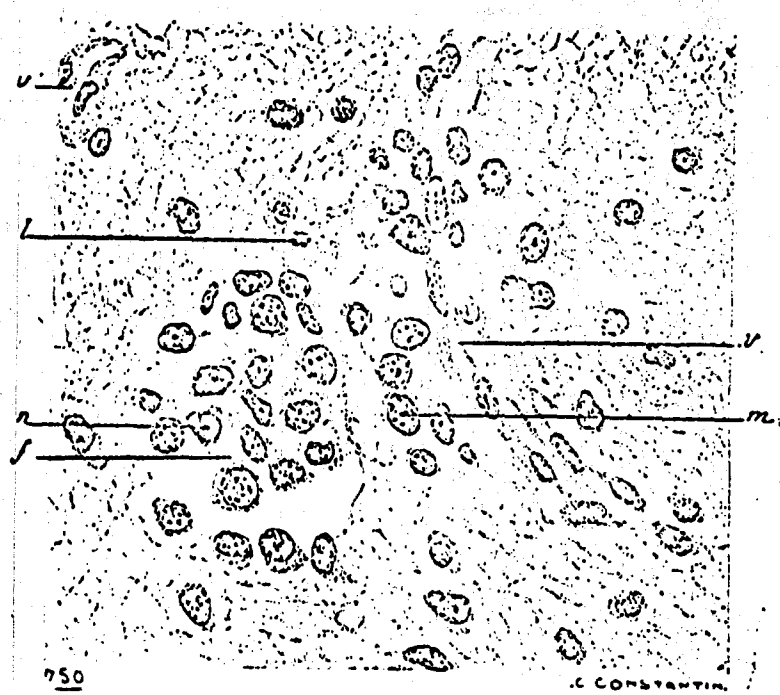

Fig. 1. — Nodule in the encephal of rabbit 89/V (Kling virus). f, nodule formed from cells of epithelioidal aspect; n, nucleus; m, mononucleare around the vessel v; l, lymphocyte; v, vessel, Hematein-eosin.

Now, these studies, finished in July 1923 and published October 20, 1923 (1), confirmed our hypothesis.

Actually, the cerebral alterations declared in the rabbits inoculated with the "Swedish virus" were certainly those described earlier by Kling and his collaborators: Meningitis with mononucleears of the cortex and of the septums, perivascular disks with lymphocytites and with plasmatic cells (on the level of the meccencephalitis) and nodules, without well defined topography. These nodules are constituted by a central zone of cells of an epithelioid appearance, with voluminous nucleus, and by a peripheral zone, rich in lymphocytic mass of necrosed cellular debris in the center of the nodule. Certain ones of these nodules are situated in

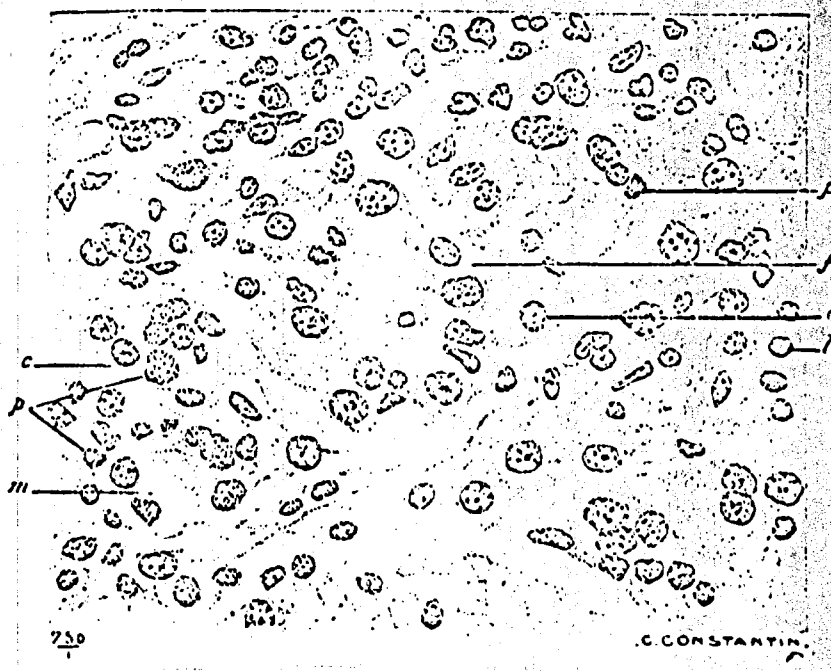

Fig. 2. — Nodule in the encephalitic of rabbit 9/T, inoculated with the spontaneous encephalo-myelitic virus of the rabbit (Twort virus). — f, nodule; l, cell of epithelioidal aspect; c, giant cell containing pigment (p); m, voluminous cell with eccentric nucleus, containing pigment; p, pigment. Hematein-eosin.

(1) LEVADITI and NICOLAU. C. R. of the Society of Biology, 89, 1923, p. 775. This note had to be presented to the Society of Biology in the last seance of July. Circumstances beyond our control retarded the publication of it

the neighborhood of an obstructed vessel (cf. fig. 1).

New alterations of the same kind were found in the encephal of two rabbits inoculated with the virus of the epizootic encephalitis, sent by C. C. Twort (fragments of encephal conserved in diluted glycerin). Here are the results furnished by these experiments:

Rabbits 9/T and 10/T. — Intra-cerebral inoculation October 6, 1928. No clinical sign apparent afterwards. Animals are sacrificed the 173th day. Histological examination of the brain shows the following lesions: chronic meningitis with mononucleears of the cortex and of the septums, peri-vascular disks under cortexes, absence of acute encephalitis, centers circumscribed (nodules) in the region of the hyppocamp. These centers are constituted by a great number of mononuclears encircling a central zone, formed by epithelioid cells containing ocher pigment. The centers touch some obstructed vessels; the neurons in the neighborhood are clearly altered in their structure (cf. fig. 2 and 3). Here and there, one distinguishes giant cells.

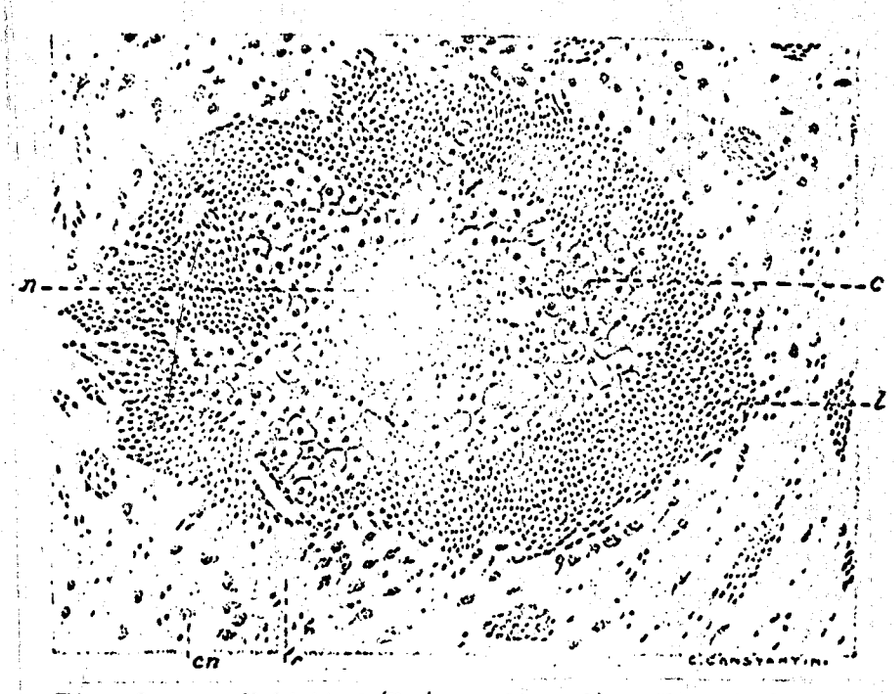

Fig. 3. — Rabbit 9/T (see fig. 2). Encephal section. — n, necrosed center of a nodule: c, cells of epithelioidel aspect; l, peripheral lymphocytic zone; c(below), white substance; cn, nerve cell. Hematein-eosin.

These lesions seem to be mistaken to the alterations declared in the rabbits inoculated with the encephalic Swedish virus of Kling and his collaborators. In addition, the animals who are stricken by it do not seem to react by apparent morbid disorders, thus conducting themselves as the rabbits infected with the Swedish germ.

It came out of these observations that these "encephaliti of the rabbit, provoked by viruses of diverse origins, human lethargic encephalitis, spontaneous encephalic-myelitis of the rabbit, general paralysis (1), have nothing in common with acute encephalitis which determines in the same animal, the encephalic virus of Levaditi and Harvier, of Doerr, of Berger, of Schnabel, or the diverse rootstocks of hermetic germs. The clinical evolution of the malady and the characteristic of the lesions are something else entirely."

Furthermore, the resemblance between the histological modifications of epizootic encephalitis on one hand, of the malady provoked by the Swedish virus, on the other hand, was at such a striking point, that there was no longer room to hesitate; the same etiological agent had to find itself at the origin of these two morbid processes, considered until then as totally distinct. From there, the necessity to discover this agent. Filterable virus, or visible microorganism? In one case as in the other, experiments of crossed immunity, or precise morphological studies had to bring a convincing demonstration.

Thus we undertook researches in this path. Between times, appeared a work by Doerr and Zdansky (2) (April 1923) regarding the same question.

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(1) PLAUT and NUIZER (Munch. med. Woch., 1922, no. 52, p. 1779) observed, in rabbits inoculated by testicular method, with emulsions of brain of general paralytics, lesions resembling those described above. After a long incubation (two to three months), the cerebrospinal liquid shows a marked pleiocytosis (despite a general normal state) and, in the encephal, one declares "lesions of the general paralysis" (Plaut), to know perivascular disks and inflammatory nodules. Total absence of Treponemas (our method, modified by Jahnel). One was struck by the ressemblance between this experimental infection and spontaneous encephalo-myelitis of the rabbit.

Recently, JAHNEL and ILLERT (Klin. Woch., 1923, no. 37, p. 1731) declared lesions ressembling those of spontaneous encephalo-myelitis in rabbits inoculated with encephal originating from a case of uremia and of another case of Wilson's malady. Cf. the works of BONFIGLIO (Policlinico, Sezione pratica, 30, 1923, p. 26).

(2) DOERR and ZDANSKY. Schweizerische. med.. Woch., 1923, no. 14.

The Swiss authors study the preparations of Kling (rabbits inoculated by cerebral method, with the "Swedish virus" of passage) and confirm his histopathological observations. They declare the presence of nodules (granulomata), whose periphery is constituted by lymphoidal elements, epithelioidal cells and giant cells, whose center is necrosed. Doerr and Zdansky shows, further, that these nodules do not exist in the nevraxe of human subjects dead of epidemic encephalitis, and that they are not present in all the brains of rabbits inoculated with encephalic materials. Of such ones granulomata can be disclosed in the encephal of animals never having been injected with encephalic virus (cf. Neuburger (1)). And to conclude: "It seems, up until a certain point, believable that this granulomatosis is a parasitic malady of the rabbit, completely independent of human encephalitis". It is worthy to remark that, in this work, Doerr and Zdansky do not establish any relationship between the malady of Kling and spontaneous encephalitis of the rabbit.

About a month after the presentation of our account to the Biology Society (October 20, 1923; November 17 and 24, 1923), appeared a long memoir of Flexner (2), on the etiology of epidemic encephalitis. In this memoir, the author, studying, in his turn, the "Swedish virus", makes some reserves on the subject of its encephalic origin. Actually, the cerebral lesions observed by Kling can be met in rabbits never having received human encephalic material, even never having been injected. Flexner recalls the observations, already mentioned, of Bull, Oliver, Dwort, and cites in particular the declarations of his collaborator Mc Cartney, who, in about 50 p. 100 new rabbits, examined at the Rockefeller Institute, reveals some alterations of chronic nevraxitis.

The memoir of Mc Cartney (3) appeared elsewhere in January 1924. It only contains confirmative documents, that which frees us from analyzing it here in detail.

The two works that have just been cited, one before, the other after ours, were thus clearly conform to our conception concerning the etiology of encephalitis provoked by the Swedish virus. However, it is the discovery of the pathogenic agent that put an end to the discussion, in demonstrating the identity between spontaneous epizootic encephalitis and the generality of encephalitis of chronic nature, provoked experimentally in the rabbit by inoculations of varied materials, of human origin or other. A few words on the facts of this discovery.

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(1) NEUBURGER. Naturforscherversammlung, Leipzig, 1922, cited according to Doerr and Zdansky.

(2) FLEXNER. Journ. of the Americ. med. Assoc., 81, 1923, pp. 1688-1735.

(3) Mc CARTNEY. Journ. of experim. Med., 39, January 1924, p. 51.

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FACTS OF THE DISCOVERY OF Encephalitozoan cuniculi, ETIOLOGICAL AGENT OF EPIZOOTIC ENCEPHALITIS OF THE RABBIT. — We have explained this history in a short note inserted in February 1924 in the Schweizerische med. Wochenschrift (1); we are reproducing it in these Annales, adding there the works since published.

In October 1923, we proposed to study, again, with the aid of further perfected methods, the histological details of cerebral lesions in rabbits inoculated with the "Swedish virus" of passage (Kling rootstock), the spontaneous encephalitis virus (rootstock Twort) and the virus isolated from human encephalitis by Thalhimer (2), in the United States. Furthermore, we would wish to compare these lesions to those that the rabbits sent from Sweden by Mr. Kling could present, to which we had inoculated, by cerebral method, the encephal of a Parisian rabbit, supposed indemnified (See p. 574). In the course of these researches, we discovered, first in the animals belonging to the Kling serie, next in the rabbits stricken with spontaneous encephalitis, or with the Thalhimer malady, finally in the Swedish rabbits, particular elements, whose parasitic nature left no doubt. In fact, the morphology, the topographic disposition in relationship with the cerebral lesions, the coloring reactions, the mode of evolution, permitted to affirm that it was there a question of a protozoan, in particular of a Microsporidia, in strict etiological relationship with the histopathological manifestations of the infection. The fruit of our studies was recorded in a serie of Communicated notes, from November 12, 1923, at the Academy of Sciences and at the Society of Biology. Here, in a few words, is the object of these Notes:

a.(1) The microorganism, which we are calling Encephalitozoan cuniculi is found in rabbits inoculated with "Swedish virus", Thalhimer's virus and in the animals stricken by epizootic encephalitis. It is the same everywhere and seems to belong to the group of protozoans. This parasite forms cysts containing spores, of which we give a precise description (November 12, 1923);

b.(2) Presence of cysts far from encephalic nodules; breaking out of these cysts and formation of granulomata, at the level of which the spores are engulfed by the macrophages; possibility of studying the parasite on smear preparation. We will consider the Encephalitozoan cuniculi as a protozoan belonging to the group of Microsporidias. Here are the conclusions which unroll from this second note: "The presence of a same parasite,

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(1) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of The Academy of Sciences, 177, 1923, p. 985, seance of November 12.

(2) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology 89, 1923, p. 984, seance of November 17.

(3) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology 89, 1923, p. 1157, seance of December 8.

Encephalitozoon cuniculi, in the encephala of rabbits stricken by the malady provoked by Kling's Swedish encephalic virus, of rabbits infected with the virus, called encephalic, of Thalhimer, and also of rabbits contaminated with the spontaneous epizootic encephalitis virus of Bull, Oliver and Twort, permits to identify these three maladies. Kling, Davide and Liljenquist, as well as Thalhimer, thus had worked with the spontaneous encephalitis germ of the rabbit, while they thought to have in their hands the virus of human encephalitis, which is filterable and invisible, as we had shown it to be from 1920. May we add that Encephalitozoan cuniculi had never been found on sections of encephals of infected rabbits, by cerebral method, with the encephalitis virus of Levaditi and Harvier, or with the herpes virus" (November 17, 1923);

C.(3) Description of kidney, cerebral, hepatica and splenic lesions. Presence of Encephalitozoan cuniculi in the kidney (on smear preparations and sections). Four illustrations show the aspect of the parasite on smear preparation and its disposition in relationship with encephalic alterations, in the rabbits stricken with spontaneous encephalitis, or inoculated with Thalhimer's virus (December 8, 1923);

d.(1) Existence of Encephalitozoan cysts on the interior of epithelial cells that cover canaliculi of the renal papillas. These cysts break and the spores penetrate in the light of the canaliculi, to be poured forth outside by the urine. The examination of the urine of contaminated animals permits to verify a variable number of spores. The propagation of the malady is made by the intermediary of these spores, which, present in the urine, soil the alimentary materials and penetrate with them into the stomach and the intestine. The spontaneous contamination seems thus to affect itself by the digestive tract. Encephalitozoan is virulent for the mouse (January 7, 1924);

e.(2) Evolution of the Microsporidia in the mouse. Morphological study of spores, their presence in the peritoneal cells and in the Kupffer cells (liver) (January 26, 1924);

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(1) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of The Academy of Sciences, 178, 1924, p. 256, seance of January 7.

(2) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology, 40, 1924, p. 194, seance of January 26.

(3) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology, 89, 1923, p. 1157, seance of December 8.

f.(3) Coloring and histochemic reactions of Encephalitozoon spores. Comparisons between the morphology of these spores and that of the Microsporidia of the snake (Glugea danilewskyi), studied by Guyenot and Naville(4). Illustrations representing the details of structure of spores of the Microsporidia and a part of its evolutive cycle (micronucleus pansporoblasts). Receptivity of the rat and of the dog. Presence of Encephalitozoon in spontaneously contaminated rats. Study of the hereditary transmission of infection in the mouse (March 15, 1924).

There are, in resume, the facts established by us on the subject of etiological relationships between Encephalitozoon cuniculi and chronic encephalitis of the rabbit, whatever be the origin of this encephalitis, spontaneous infection or experimental inoculation.

* * *

What was known of this parasite before the publication of our first Note at the Academy of Sciences (November 12, 1923)? A single work, relating to encephalitis provoked in the rabbit by the Kling virus, and containing a few indications on the subject of the presence of particular formations in the encephal, had appeared in April 1923; it was signed by Doerr and Zdansky (loc. cit.). These authors stained with intense stain by the Ziehl-Neelsen method the sections of brain that Kling had sent to them, and they there discovered corpuscles, whose microbic nature, far from seeming certain to them, was only, in their opinion, at the very most possible. Here is the description that they give of these corpuscles:

"Long formations of 1.5 to 3 microns, colored in red, of variable aspect, are accumulated in the epithelioidal cells which occupy the center of the nodules, and above all in the necrotic zone of these nodules. Beside the egg-shaped or elongated corpuscles, one finds other ones which appear paler at the two extremities, and still other ones that are incurved. One can verify a nuclear formation be it in the center of the corpuscle, or near one of the poles."

Doerr and Zdansky verify these corpuscles in the protoplasma of epithelioidal cells. Analogous formations had been found in the encephal

(3) LEVADITI, NICOLAU and Miss SCHOEN. C. R. of the Society of Biology, 40, 1924, p. 662, seance of March 15.

(4) GUYENOT and NAVILLE. Swiss Review of Zoology, 30, 1922, no. 1.

of a rabbit that had been inoculated with a rootstock of encephalic virus "Basel III", by intra-cerebral method, and which had been sacrificed four months later.

Thus it was a question of corpuscular formations appearing to offer a certain structure and lying in the middle of granulomatas. No cystic disposition is mentioned in this work; it is a question neither of the presence of the microorganism far from the nodules, in full cerebral substance, nor of the least morphological detail observed in smear preparation. Further more, the utilized methods of coloration (hematein-Eocene at first, Ziehl-Neelsen) next, showed that the formations in question were clearly of an acid-fast character. As for the interpretation that Doerr and Zdansky accorded to the corpuscles observed by them, here it is textually:

"The corpuscles described could (1) be microorganisms, opinion shared by several specialists to whom we have shown our preparations last December. But a quite particular prudence imposes itself, above all when it is a question of declarations concerning the central nervous system, and principally when one utilizes the methods of coloration which put in evidence fatty elements (acid-fast characters). Besides, we have not had the occasion to realize all of the desirable control researches and to utilize procedures of coloration permitting to formulate a clear opinion. The important thing is to know if the granulomatas and the described corpuscles (it is still necessary to know if these last ones are parasites, falls es sich um Parasiten handeln sollte) are in relationship with the etiology of encephalitis."

It results from it that if Doerr and Zdansky, saw on Kling's sections the more or less altered spores of Encephalitozoan cuniculi, they gave but one inexact description of their tinctorial affinities, since they talk of acid-fast character, which is contrary to reality. Further more, they did not affirm, with all of the certitude desirable in a similar occurence, their parasitic nature, still less the characteristics which make of them protozoans belonging to the group of Microsporidies.

It is only on December 27, 1923, more than a month after the publication of our Note to the Academy of Sciences, and when our three first communications had already appeared, that Doerr and Zdansky (1), returning to the question, confirm the microbic nature of formations observed in April 1923. This time, they describe the cysts, of which they give an illustration, and realizing that the acid-fast character, mentioned in their first work, is nothing less than certain. Being given that all this was demonstrated by our former works, one asks himself for what reason

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(1) Underlined in the text.

(1) DOERR and ZDANSKY. Schweizerische med. Woch., No. 52, 27th of December 1923, p. 1189.

Doerr and Zdansky pretend to describe, in this second Memoir, a "new parasite". (Heute konnenwir uber einen NEUEN PARASITEN berichten, etc.).

* * *

Our researches were nearly finished, when we found, in a recent publication of Mc Cartney (loc. cit.), a bibliographical indication of higher interest. It concerns a work of J. Wright and E. Craighead (1), appeared in July 1922 and concerning the study of an infectious driving paralysis of young rabbits. These authors tried to transmit infantile paralysis to rabbits, without success elsewhere, and, in the course of these tentatives, observe a spontaneous infection manifesting itself by drowsiness, tremblings, paralysis, and terminating itself often by death. The nervous system of these young animals show inflammatory and necrotic lesions, in the neighborhood of which one declares corpuscles having the aspect of illustrated elements. These corpuscles contain one or two light vesicles, are 4 microns in length and 1.5 microns in width, are colored by the Gram and have a relative acid-fast character. Further more, Wright and Craighead declare the same formations in the renal cells, in the light of canaliculus of the kidney and also in the urine of infected animals. The authors conclude that it concerns, in the species, a microorganism belonging, very probably, to the group of protozoans, in etiological relationship with the spontaneous malady of young rabbits, malady of which the propagation would take place by the intermediary of the urine.

The comparison of microphotographies that illustrate the work of Wright and Craighead and of our preparations show a striking ressemblance between Encephalitozoon cuniculi and the microorganism observed by the American authors. Everything brings one to believe that epizootic paralysis of young rabbits is only a particular form of spontaneous encephalic-myelitis, studied by Bull, Oliver and Twort, and that the etiological agent is the same in the two morbid processes.

If, in the future, the hypothesis of the identity between the paralysis of the young rabbits and spontaneous epizootic encephalitis found itself confirmed, we would regret that at the Encephalitozoan cuniculi denomination, proposed by us to designate the etiological agent of epizootic encephalitis, the names of Wright and Craighead could not be added. The Microsporidia that provokes encephalic-myelitis of the rabbit had to be called Encephalitozoan cuniculi (nov. spec.) [Wright and Craighead].

(1) WRIGHT and CRAIGHEAD. Journ. of experim. Med., 36, 1922, p. 185.

CHAPTER II

EXPERIMENTAL STUDY

I. ENCEPHALITOZOAN CUNICULI IN RELATIONSHIP WITH THE "SWEDISH VIRUS". __ General remarks. __ Experimental study of the etiological role of Encephalitozoan cuniculi implies the two following reserves:

1 The existence of a epizootic infection in the rabbit, infection whose frequency seems to vary following the regions and the rearings, fact that in every tentative of transmission by inoculation one must keep in mind the possibility of a spontaneous contamination of supposedly fresh animals. Luckily our stocks of rabbits, originating from the Parisian region have shown themselves to be exempt from epizootic encephalitis except for a few very rare exceptions. In fact, on nearly 700 encephals examined on smear preperation and on sections since the discovery of encephalitozoon, we have only met three of them showing characteristic lesions, as well as parasites. It results that the causes of error in the interpretation of our results are reduced to the strictest minimum. It is not of the same results in experiments on the mouse, animal in which the cerebral Microsporidiosis is infinitely more frequent, as we will see later on.

2 The evolution of spontaneous encephalitis, or of chronic encephalitis provoked experimentally, is of the slowest (Kling and his collaborators). Slow also is the cerebral development of the Encephalitozoan. It is generally necessary at least one and one half months to two months in order that the encephalic alterations become appreciable and that the parasite can be disclosed on the smear preparation and on the sections. It follows that, if one practices inoculations on a great number of rabbits, it is necessary to keep track only of the results furnished by the animals that survived beyond fifty to sixty days.

We would conform strictly to these indications in the interpretation of our experimental data.

The Protocol I (see Annex) shows that, among the rabbits inoculated with Kling's Swedish virus (rootstock Henriksson, seventh generation + rootstock Karl, I E, second generation, of October 6, 1922, conserved in diluted glycerin), two presented intense lesions of the brain and also parasites disclosable on sections. One of these animals was sacrificed one hundred five days after the inoculation; the other had been examined the one hundred fourteenth day.

The character of microscopic modifications and the morphology of Encephalitozoon correspond to the particularities of the same lesions and parasites declared in the rabbits stricken with spontaneous encephalitis (cf. Plate III, fig. 2 and 7).

II. Encephalitozoon cuniculi IN RELATIONSHIP WITH C. C. TWORT'S SPONTANEOUS ENCEPHALIC-MYELITIS OF THE RABBIT, ENGLISH ROOTSTOCK). The first glycerined virus sent by Mr. Twort served at the intra-cerebral inoculation of rabbits 9/T and 10/T, of which the clinical and anatomical-pathological observation was exposed page 662. In both of them, Encephalitozoon was found on the level of encephalic nodules.

Further more, in December 1923, C. C. Twort was kind enough to confer upon us in London one of his spontaneously contaminated rabbits. The brain of this animal (discreet lesions) was inoculated, in the fresh state, in 6 rabbits, sacrificed or dead from the forty-second to the one hundred second day, 5 offered parasites. These last mentioned were present, now in the encephal, now in the kidney. Two rabbits had a parasited nevraxe, while three others showed cysts in the renal epitheliums.

The English virus thus seems virulent for the rabbits of the Parisian region (1).

III. Encephalitozoon cuniculi IN RELATIONSHIP WITH THALHIMER'S VIRUS (2). Thelhimer (Milwaukee) inoculates rabbits with materials originating from human encephalitis cases (liquid cerebrospinal), and declares some lesions of the nevraxe ressembling alterations of epizootic encephalitis (meningitis, peri-vascular and nodule disks). The author however was persuaded, like Kling and his collaborators, to have experimentally transmitted lethargic human encephalitis to the rabbit.

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(1) Concerning this, we want to attest that C. C. TWORT saw Encephalitozoon cuniculi before the first publications of DOERR and ZDANSKY and of LEVADITI and his collaborators. In fact, at the time of our voyage to London, in December 1923, C. C. TWORT showed us sections of encephal containing parasitic cysts. Convinced of the filterability of the spontaneous encephalic virus, C. C. TWORT had considered these cysts as a secondary infection, without etiological reletionship with the maledy.

(2) THALHIMER. Archives of Neurology and Psychiatry, 5, 1921, p.113; 8, p. 286.

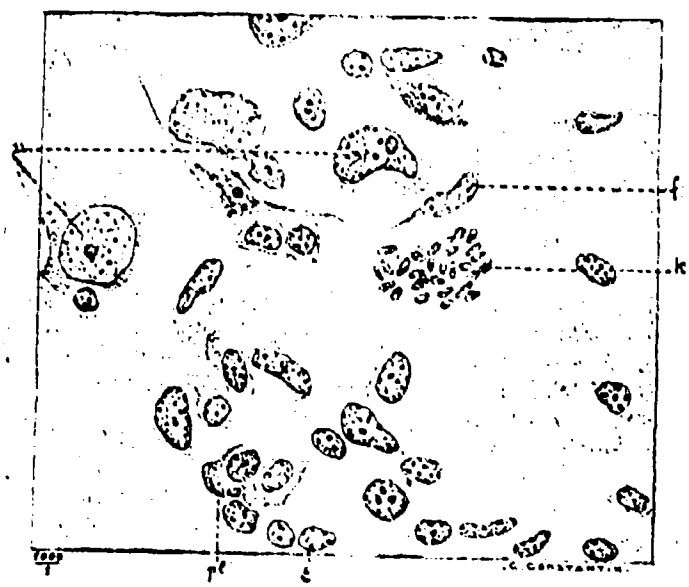

Fig. 4. __ Cerebral nodule in rabbit 6/Y. Thalhimer Virus. n, nerve cells; pl, plasmatic cells; f, fusiform cell; k, cyst containing Encephalitozoan. Giemsa coloration.

Mr. Thalhimer was kind enough to send us several samples of his virus, conserved in diluted glycerin. The inoculation of this virus in the rabbit furnished us with results consigned to Protocol III. One sees there that 2 rabbits, 6/Y and 56/V, inoculated by cerebral method with rootstock Thalhimer 400 __ 4 __2, were sacrificed the one hundred fifty-seventh day. Both of them presented cerebral and mesocephalic alterations, consisting in meningitis with mononuclears, peri-vascular disks and nodules containing cariolyzed polynucleers. Typical parasites were distinguished in the encephal of the rabbit 6/Y (cf. fig. 4).

It results from it that Thalhimer, like Kling and his collaborators, believes to have transmitted to his rabbits human encephalic virus, while actually he was in the presence of the epizootic encephalic germ.

IV. __ Encephalitozoan cuniculi, ETIOLOGICAL AGENT OF EPIZOOTIC ENCEPHALITE OF THE RABBIT, PARTICULAR ROOTSTOCK.

A. __ INOCULATION BY INTRA-CEREBRAL METHOD

a) Our first experience of transmission was made with Parisian virus on rabbits of Swedish origin (Protocol IV), besides, completely unknown to us. In fact, persuaded since July 1923 that the encephalitis provoked by Kling and his collaborators was due to the localisation of the spontaneous encephalic virus on the nevraxe, still unknown at this epoch, localization facilitated by a traumatism of the nervous system, we proceeded in the following manner:

From the stock of 12 young Swedish rabbits sent by Mr. Kling, we selected 10 of them, which we inoculated, by cerebral method, from fragments of encephal of a Parisian rabbit supposed fresh and that had just been sacrificed (rabbit 30/V). We had thought thus to realize this traumatism of the brain, destined to make the tension center. The 10 rabbits were left alive until the beginning of October 1923, and it is then only that we examined the brain of rabbit 30/V, considered indemn. Now, we discovered there, not only the characteristic lesions of spontaneous encephalitis, but still Encephalitozoon cuniculi cysts (see fig. 5 and 6).

This examination showed to us thus, afterwards, that in reality the 10 Swedish rabbits had been inoculated, not with a normal emulsion of encephal, but with a suspension of nevraxe containing the germ of spontaneous encephalitis, Parisian rabbit tock.

The result of this first experiment is recorded in Protocol IV. One sees there that, among the 10 inoculated rabbits, dead or sacrificed from the eighty-third to the two hundred forty-eighth day, two only were exempt from cerebral lesions (rabbits 31/V and 39/V, sacrificed the eighty-third and the two hundred forty-eighth day). They seem to have escaped from the infection until then. A third rabbit (rabbit 32/V, de . the eighty third

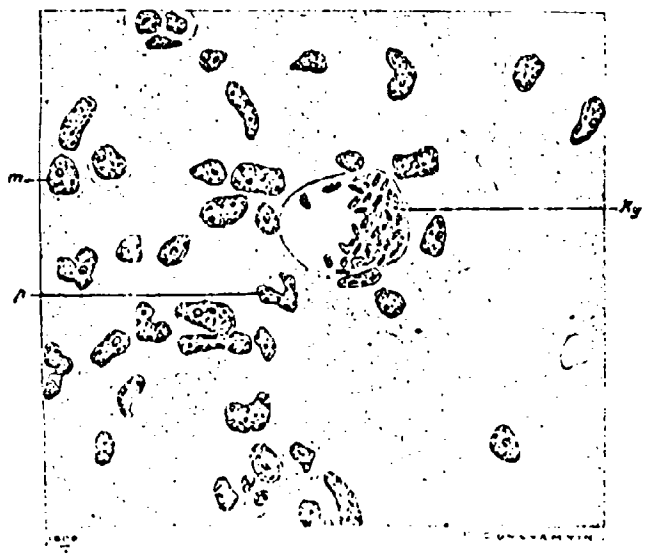

Fig. 5. — Rabbit 30V, stricken with spontaneous encephalitis, Parisian rearticol. Brain section. m, mononuclear; n, polynuclear; beginning of reaction around an Encephalitozoon cyst (Ky); on the wall of the cyst, a cell with flattened nucleus. Mann method.

day), offered encephalic alterations, without detectable parasites on the sections. On the other hand, in the 7 other animals, we observed lesions and Encephalitozoon, be it in the brain, or in the kidney, or in the two organs at the same time. There was thus infection in 70 p. 100 of the cases. Here are the results obtained, following the examined organ:

Brain: 7 positive cases on 10 . . . . . 70 p. 100
Kidney: 4 positive cases on 7 . . . . . 58 p. 100

One must note that, sometimes, one can declare cerebral alterations noticeable, without being able to notice ENCEPHALITOZOAN on the sections. That is understandable, if one takes into account the two following considerations:

First, the microorganisms being deposited by groups, it is necessary to examine quite a large number of preparations before discovering a cyst, or parasited nodules. It is possible thus that such examinations rest negative, despite the real presence of the microbe in the encephal.

In the second place, the nodular alterations being the expression of a defense reaction with regard to the encephalic parasite, one conceives that at a given moment these reactions end in the more or less total destruction of the germ. This is what happens quite frequently in the kidney, as we will see in the course of this Memoir.

b) In a second serie of experiments (Protocol V), an emulsion prepared

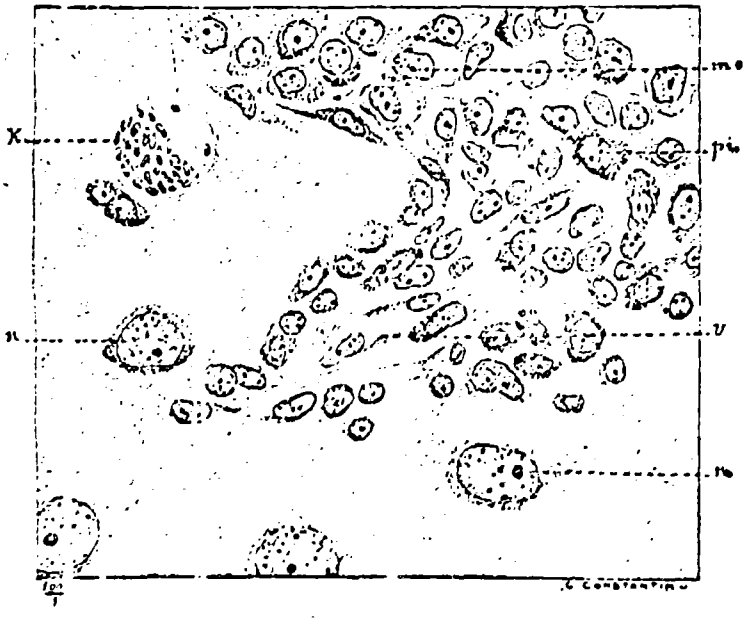

Fig. 6. — Cerebral nodule in Rabbit 30 V (see fig. 5). Spontaneous encephalitis. m. big mononuclears; X, vessels; pi. pigmentary cell; n. nerve cell; k. cyst containing Encephalitozoon. Eosin-orenge-blue of Unna.

with the encephals of rabbits 34/V, 35/V and 41/V, containing Encephalitozoons, was inoculated into 5 rabbits, by cerebral method or by peritoneal method. These ones are dead or were sacrificed from the seventy-fifth to the one hundred forty-seventh day. In 3 of them, the Encephalitozoon was found either in the brain, or in the kidney, or still yet in the two organs simultaneously. The frequence of the positive inoculations was from 60 p. 100.

c) The encephals of rabbits 36/V, 76/U and 40/V, enclosing parasites, served to prepare an emulsion that was injected in the brain of four fresh rabbits; these were dead or were sacrificed from the 76th to the 132nd day. In three of them, we noticed Encephalitozoon in the brain or in the kidney. The percentage of positive inoculations was from 75 p. 100 (See Protocol VI).

d) The results were less favorable in a fourth series of attempts (inoculation in 6 rabbits of a mixture made with the brains of rabbits 13/V and 37/V, both of them infected). Among these (dead or sacrificed from the 110th to the 125th day), only one proved to be contaminated [rabbit 93; presence of Encephalitozoon in the kidney (See Protocol VII)]. Percentage of positive inoculations: 16 p. 100.

These attempts show that intra-cerebral inoculation of encephalic emulsions containing Encephalitozoon cuniculi confers the malady to the rabbit, little matters the race of animals utilized (Swedish rabbits or rabbits of the Parisian region). The frequence of positive results can vary from one serie to the other. In three of our experiments, this frequency oscillated between 60 and 75 p. 100, but, in a fourth trial, 16 p. 100 only of inoculated rabbits showed themselves to be parasited. These deviations are attribuable, on the one hand, to the richness in germ of the injected material, on the other hand, to the more or less pronounced receptivity of the animals in experiment.

It is interesting to state that despite the exclusively intra-cerebral inoculation of the virus, this one can localize itself in the kidney without the encephal being apparently parasited.

It was impossible for us to precise the reasons of this preference of the germ for the kidney or for the brain, but the fact rests no less incontestable: in certain rabbits, one of these organs shows itself more apt than the other to attract the Encephalitozoon and to react by more or less pronounced histological alterations.

e) Positive results were obtained by inoculation of parasited renal emulsions in the encephal of fresh rabbits. Protocol VIII shows that, among the four animals infected in this manner, with a virus originating from rabbits 36/V, 76/U and 40/V, and that are dead, or were sacrificed

from the 71st to the 132nd day, three contracted the malady (75 p. 100). The Encephalitozoon was discovered in the urine, in the kidney and in the encephal. In addition, in the experiment that is the object of Protocol IX, one of two rabbits inoculated in the brain with a parasited renal emulsion, originating from rabbit 37/V (examined, one the 75th, the other the 112th day), was contaminated (presence of germs in the kidney). An analogous result was registered in the course of trials consigned in Protocols X and XI. There results from it that at the example of the encephal, the kidney can serve for the transmission of the infection by intra-cerebral inoculation. The frequence of positive results seemed to equal that which one observes when one utilises the emulsions of parasited brains in intra-cranial injection.

B. __ INOCULATION IN THE SCIATIC NERVE.

A kidney emulsion of rabbit 42/V, containing numerous Encephalitozoon, was inoculated in the sciatic nerve of rabbits 35/A and 36/A. The first of those animals died the 103rd day; the encephal and the kidney were parasited (1) (see Protocol XI bis). The second animal was sacrificed the 116th day; absence of Encephalitozoon in the brain and in the kidney.

This experiment shows that it is possible to transmit encephalitis epizootic to the rabbit by inoculation of virus in the sciatic nerve.

C. __ INOCULATION BY INTRA-VEINOUS METHOD

The intra-veinous method seems to lend itself to the experimental transmission of epizootic encephalitis. An experiment, resumed in Protocol XII, shows that the four rabbits injected in the marginal vein of the ear, with a kidney emulsion originating from rabbit 42/V (presence of Encephalitozoon) and that died, or were sacrificed from the 59th to the 111th day, were contaminated. In two of these animals, Encephalitozoon was distinguished only in the encephal, while in the two others parasites were declared in the brain or in the kidney.

This trial proves that the infection is transmissible by inoculation of virus in the circulatory stream (see Protocol XII).

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(1) Absence of parasites and of lesions in the inoculated sciatic nerve.

D. __ INOCULATION BY INTRA-TESTICULAR METHOD

We inoculated in two rabbits, by intra-testicular method, an emulsion of encephal and of kidney originating from rabbit 71/B, containing some Encephalitozoan (Protocol XIII). The first of these animals died the 57th day, the second succumbed the 54th day. In one, as in the other, we found parasites in the kidney. Nevertheless, the examination of the testicles, practiced on sections as well as on smear preparations, revealed neither appreciable alterations, nor Encephalitozoan.

This experiment shows that microsporidian infection is transmissible by inoculation of virus in the testicular tissue; it seems to overcome the kidney, without localizing to begin with on the seminal gland.

E. __ INFECTIOUSNESS OF THE PERITONEAL LIQUID.

In a certain number of our rabbits, contaminated experimentally, we verified a quite marked ascites. The examination of the peritoneal liquid brought to light rare lymphocytes, but it was impossible for us to find Encephalitozoan spores there. The presence of the ascites explains itself by the existence of renal lesions, so frequent in the course of epizootic encephalitis.

Ascites liquid was injected, by cerebral method, in a rabbit (Protocol XIV). This rabbit (89/B) was sacrificed the 132th day, without being visibly parasited.

The peritoneal liquid does not seem to close in the microsporidian germs, discernable on smear preparation, or by inoculation in fresh animals.

F. __ INFECTION BY CONTACT

It was interesting to establish if the rabbits placed in the same cage as the experimentally infected animals were susceptible to contracting epizootic encephalitis. Two experiments of this kind were realised (cf. Protocols XV and XVI).

In the first one, we put into contact two Swedish rabbits 41/V and 42/V, with the great series of 10 animals having received, by cerebral method, the virus of spontaneous encephalitis, Parisian rootstock (cf. Protocol IV). These two rabbits lived in contact with the others during 165 and 147 days. They were then sacrificed. One of them (rabbit 41/V) showed neither lesions, nor Encephalitozoan. The other (rabbit 42/V)

offered intense alterations of the brain and of the kidney, with the presence of quite a large number of parasites. These same parasites had been noted in the urine.

In a second serie of trials, two fresh animals were placed in the same cage as the contaminated rabbits 36/V, 37/V and 42/V. The first of these rabbits died the 60th day, without lesions or parasites. The second one was sacrificed the 119th day. Its brain as well as its kidney presented obvious alterations and some Encephalitozoon.

There results from it that the rabbits that live during a quite prolonged time (119 to 147 days) in contact with the animal carriers of Encephalitozoon contract the infection. The latter finishes by localising itself on the encephal and on the kidney (elimination of the germ by urinary secretion).

C. __ INFECTIOUSNESS OF THE URINE.

We verified, many times over, the presence of spores of Encephalitozoon in the urine, gathered together in vivo, by pressure on the bladder, or post mortem, by vesical puncture. The smear preparations, made with culot obtained by centrifugation of the urinary secretion, showed plate epithelial cells, granulous cylinders, absolutely typical leucocytes and spores. The experiment permitted to establish that these spores, present in the urine, were capable of germineting and of confering encephalitis to fresh rabbits (cf. Protocol XVII).

In one of these experiments, some urine, gathered up post mortem in two rabbits whose kidneys were parasited, was inoculated, by cerebral method, into rabbit 91. This urine contained spores. This rabbit was sacrificed the 123rd day; its brain as well as its kidney contained Encephalitozoons; these organs were obviously lesioned, besides.

One can transmit the infection in administering the urine, not only by cerebral method, but quite simply per os. Thus, in one of our trials, urine collected in vivo in rabbit 42/V [presence of parasites in the kidney(1) and in the urinary secretion] was administered two times, by the stomachic probe, to rabbit 190 (see Protocol XVIII). The animal was sacrificed the 109rd day. Its kidney, as well as its brain were strongly altered and contained Encephalitozoons.

These trials show that the urine of experimentally infected animals, or spontaneously contaminated, can enclose Encephalitozoon spores; however, it is virulent when it is administered to fresh rabbits, either by intra-cerebral method, or by stomachic method.

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(1) The kidney was examined later, when the rabbit was sacrificed.

Such results are very favorable to the hypothesis of natural transmission of epizootic encephalitis by gastro-intestinal method. The germ, multiplying in the kidney, eliminates itself by renal canaliculus (see Chapter III), invades the urine and thus contaminates the foods. The spores, deposited on these foods, are swallowed at the same time as they are, then, by a still imprecise mechanism, succeed in crossing the barrier that opposes them the buchu-pharyngeal and gastro-intestinal mucous membranes. Do they germinate in the intestine itself? Are they englobed by the leucocytes that transport them elsewhere? So many problems that rest to be solved.

II. __ IS THE VIRUS OF SPONTANEOUS ENCEPHALITIS A FILTERABLE VIRUS?

In the beginning of this Memoir we saw that, according to Kling and his collaborators, the so called "Swedish" virus would be capable of traversing the filter candles in porcelain. In the species, it would be a question of a filterable virus, similar to the eltra-virus of encephalitis (Levaditi and Harvier), of the herpes (Luger and Lauda), of the poliomyelitis (Landsteiner and Levaditi), etc. On his part, C. C. Twort, basing himself on simple analogies, envisaged, he himself, the virus of epizootic encephalo-myelitis as a germ belonging to the group of invisible and filterable microorganisms.

Despite the microsporidian nature of the etiological agent, demonstrated by our observation, we experimentally researched to see if this agent was capable of traversing the Chamberland candles nos. I and III. The dimensions of the Encephalitozoan spores render very little probable their filterability. It is possible however that the Microsporidia comprises, in the course of its evolutive cycle, unsuspicious forms, small enough to cross through the filters. What does the experiment show on this subject?

We prepared a cerebral emulsion rich in Encephalitozoan, that we first centrifuged, then filtered under pressure through a Chamberland candle no. III (sterile filtrate, see Protocol XIX). The filtrate was inoculated by intra-cranial method, into four rabbits, that were sacrificed from the 84th to the 128th day. Not one of them presented lesions or Encephalitozoans.

In a second series of experiments, the filtrate (Chamberland candle no. I), prepared from two encephals containing parasites, was administered, by intra-cerebral method, to 7 rabbits. These died or were sacrificed from the 102nd to the 111th day. The result was similar to the preceding: total absence of microscopic alterations and of parasites, in the brain as well as in the kidney (cf. Protocol XX).

One can conclude from these different researches that the Encephalitozoon cuniculi is not composed of viable forms, capable of passing through filter candles.

I. __ VIRULENCE OF THE Encephalitozoon cuniculi For ANIMAL SPECIES OTHER THAN THE RABBIT.

1 Guinea pig. __ Our experiments on the guinea pig are too few to permit definitive conclusions (see Protocols XXI, XXII AND XXIII). In animals infected by cerebral or peritoneal method, and who survived from fifteen to forty-one days, we found neither lesions, nor parasites in the kidney or brain. It was the same thing in guinea pigs sacrificed the 108th day; however, in one of these last animals, it seemed to us that a microsporidian was detectable on the smear preparation kidney.

2 DOG. __ A dog was inoculated, by intra-cranial method, with a cerebral emulsion of mouse containing numerous Encephalitozoons. The animal succumbed the 22nd day. One verified, at the necropsy, an intense congestion of the meninges and of the brain. Quite a few spores were discernable on the smear preparations of encephalitis (see Protocol XXIV).

3 MONKEY. __ A cerebral emulsion of mouse, rich in Encephalitozoon, was injected in the brain of a Macacus cynomolgus. The animal, sacrificed the 32nd day, showed neither microscopic alterations, nor cysts or microsporidian spores (see Protocol XXV).

4 RAT. __ Control researches assured us, first of all, that rats originating from the same rearing of the Pasteur Institute that furnished our rats do not seem subject to a spontaneous infection by the Encephalitozoon cuniculi (1). In fact, the examination of the brain of twelve fresh rats showed negative, on the smear preparation as well as on the sections.

In a first series of experiments (see Protocol XXVI), four rats were inoculated by peritoneal method, with an emulsion of rabbit brain containing quite a few Encephalitozoon spores (rabbit 42/V). These animals died from the 22nd to the 67th day. Two proved to be contaminated, namely: rat 1, dead the 22nd day (presence of microsporidian spores in the peritoneal cells (see page 699), and rat 2, dead the 56th day(parasites on

(1) It is the same in the guinea pig (nine negative results on nine examinations).

smear preparation of encephal).

Same result in a second series of trials. This time, we injected in the peritoneal cavity of four rats a kidney emulsion originating from the same rabbit 42/V (presence of Encephalitozoan). The animals died from the 31st to the 49th day. Rat 1 showed typical parasites in the liver; rat 2 offered, on smear preparation, spores localized in the encephal (see Protocol XXVII).

The rat is thus susceptible to contract the infection by injection of virus in the peritoneal cavity.

5 MOUSE. — In the course of our researches on the transmission of spontaneous encephalitis to the mouse, we examined sections of brain of a mouse originating from the rearing of the Pasteur Institute, and which had never been inoculated. We there declared the presence of encephalitis lesions and of typical microsporidian cysts. Later on, we sacrificed 7 mice having lived in contact of our contaminated animals and 7 others originating directly from the Pasteur rearing. The first series comprised 5 parasited mice; 3 of the animals of the second serie offered Encephalitozoons localized in the encephal.

It resulted from these first examinations that the mouse is subject to enzootic spontaneous encephalitis, provoked by a Microsporidia offering all of the characteristics of Encephalitozoon cuniculi. Between time, appeared a work by Cowdry and Nicholson (1) ending up in the same conclusions as ours. The authors find, in 25 mice on 141 examined at the Rockefeller Institute, chronic encephalitis lesions ressembling the alterations described in the rabbit by Bull, Oliver and C. C. Twort. Moreover, they there discover parasites (spores of 1.8 to 2 microns long by 0.5 to 0.8 microns wide; cysts) that they compare to the Encephalitozoan cuniculi.

These declarations show that the mouse is frequently infected by a virus of apparently the same nature as the etiological agent of enzootic encephalo-myelitis of the rabbit. In what proportion? All depends, very logically, of the rearings which furnish the animals, and also from the precise moment where the mice of the same origin are examined. After our investigations, on 37 animals of normal appearance, 26 presented encephalitozoon in the brain, as well as more or less pronounced lesions, be it a percentage of 70 0/0.

One conceives that the frequency of the spontaneous malady in the mouse renders difficult, if not impossible, experimental study of the infection on this species of animal. Also, we are embarrassed to formulate

(1) COWDRY and NICHOLSON. Journal of the Americ. med. Assoc., 82, February 4, 1924, p. 545.

no matter how precise it be on the subject of results furnished by numerous trials undertaken on the mouse, in the goal of elucidating diverse problems, such as, for example, the filterability of the virus, its methods of penetration, the mode of contagion, etc. Let us say, simply, that the inoculation of the most varied virulent materials (brain, kidney, urine, peritoneal liquid, dried out virus, virus conserved in glycerin, etc.), practiced on 92 mice, furnished 56 clearly positive results, let us say on a percentage of 60.8 p. 100.

In order to precise if the infection is hereditarily transmissible in the mouse, we examined the encephal of a great number of descendants aged from two to thirty days, issues of injected mothers and who had lived in contact with the mothers. All of the examinations stayed negative (smear preparation and sections, see Protocol XXVIII). A single small mouse, fifteen days of age, on the 37 animals in observation, was parasited, among its brothers and sisters belonging to the same litter. It is strongly probable that this little mouse infected himself in contact with the mother.

These declarations, as does the absence of Encephalitozoon in the ovary and the testicle of rabbits and of mice stricken with spontaneous encephalo-myelitis, renders hardly believable the hereditary transmission of the infection(1).

Conclusions.

The ensemble of data exposed in this chapter shows that the Encephalitozoon cuniculi can be present, either in the encephal, or in the kidney, or still yet simultaneously in these two organs, in rabbits inoculated with the viruses of Kling and of Thalhimer, or experimentally infected with the epizootic encephalo-myelitis virus. The brain seems more frequently parasited than the kidney (66 p. 100 in place of 33 p. 100).

In addition, the infection is transmissible to the rat, to the dog and to the mouse. This last animal species is subject to an epizootic

(1) We did not discover parasites in the placenta and the embryos of a rabbit stricken by epizootic encephalitis (experimental inoculation). Since the editing of this Memoir, we examined more closely the question of the contaminations of descendants issues of parasited procreators (mouse). Certain litters are infected, while others can be indemn. We will return to this subject later on.

encephalitis provoked by a microsporidia resembling an Encephalitozoon cuniculi., malady that seemed to transmit itself by contact and which does not seem hereditary.

In the rabbit, the virus eliminates itself by urinary secretion. Spontaneous contamination effectuates itself by the intermediary of foods which contaminate the urine, rich in microsporidian spores. Penetration of the germs in the organism seems to operate through the nasal-pharyngeal and gastro-intestinal mucous membrane.

CHAPTER III

MORPHOLOGICAL STUDY OF THE ENCEPHALITOZOAN CUNICULI

The morphological characteristics of the Encephalitozoon cuniculi were defined on the smear preparation and on the sections.

1. __ SMEAR PREPARATION METHOD. __ Technique: Fixation of dried out smear preparations, by Bouin-Brazil liquid, from twenty minutes to two hours, water bath; minutes in absolute alcohol, water bath. Coloration;
a) Orange G (1 p. 100) for ten minutes, water bath;
b) Eosin (1 p. 100* for twenty to thirty minutes, water bath;
c) Blue polychrome of Unna (1/10) for fifteen to twenty minutes; water bath. Differenciation by absolute alcohol added to essence of clove. Absolute alcohol, xylol, mounting in balsam.

a) Examination in the fresh state: One takes up a small fragment of cerebral skin, that one breaks up between the slide and the glass cover, after addition of a few drops of dirty isotonic water. The examination permits to discover microsporidian spores, refractive corpuscles, oval or lightly pear-shaped, without structural details. These spores are immobile and are not colored by the blue of the methylene(vital coloration) (1).

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(1) It was impossible for us to provoke the departure of the germ from the filament, in making the diluted acids react on the fresh preparations. Besides, the observation is rendered difficult by the result of the opacity of the medium (cerebral or renal emulsion).

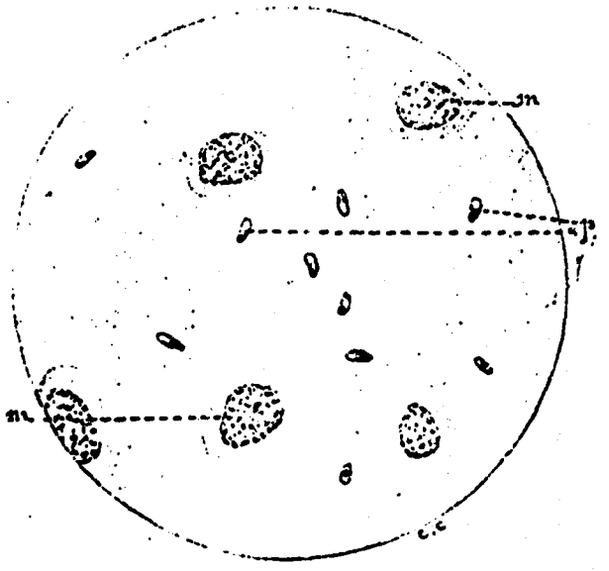


Fig. 6. __ Smear preparation of rabbit brain stricken with spontaneous encephalitis. __ m, mononuclear cells; n, Encephalitozoon cuniculi. Eosin-orange-blue polychrome of Unna. Bulk: 1/1000.

b) Examination after coloration: Certain smear preparations of brain have the appearance of a rich culture of microsporidias.

One meets with, on each microscopic field, 2-5 to 20 isolated spores, or deposited by groups. The aspect of these spores is the following: the corpuscle is delimited by a membrane, contains a biconcave disk of chromatin, deposited transversally, situated nearer one of the two poles and separating the two polar vacuoles. These vacuoles are of unequal dimensions; the great vacuole is situated near the the least drawn out extremity of the spore. In this vacuole one distinguishes a grain of chromatin, appearing attached to a thin filament (see. fig. 6 and 7; Plate IV, fig. 9).

Dimensions: longitudinal = 2.5 microns; transversal = 0.5 microns by 1 micron.

c) Coloring and histo-chemical reactions: The microsporidian spores, arrived at a state of maturity, do not color on smear preparation (brain of mouse or of rabbit), by the Panchrome of Laveran, or by the prolonged Giemsa, after fixation in absolute alcohol. Only the young forms (sporoblasts) color in a deep violet by these procedures. The envelope of the

spore seemed impermeable to the basic coloring materials, as well as to iron hematoxylin. The previous fixation of the Bouin-Brasil smear preparations modifies the permeability of this membrane and renders the spore colorable by the orange-blue polychrome eosin of Unna or by leMann. With this last technique, the spore appeared tinted in bright red, on the blue background of the preparation (analogy with the Negri bodies) (see Plate IV, fig. 9).

On the example of Guyenot and Naville (1) (study of the microsporidia of the snake, Glucer Jonilewskyi), we modified the permeability of the spores' membrane, in treating the smear preparation (before all fixation) by the normal sodium carbonate, pure chlorhydric acid and sulfuric acid at 5 p. 1,000 (two to four minutes). This preliminary treatment facilitates the colorability of the spores by iron hematoxylin and the precision of certain structural details. Let us add that the coloration methods of the bacterial spores (mordant action with chromic acid, coloration by carbol fuchsin) stay without effects on the spores of the Encephalitozoon. These spores are not acid-resistant.

d) Details of structure: The spores (smear preparation of mouse brain), treated first of all with chlorhydric acid, colored next by iron-hematoxylin, show the structural details represented by figure 8. The spore closes in one or two chromatic granulations (nuclei), situated in the vacuole corresponding to the posterior pole (d, e, i). The most frequent aspect is the one drawn in i. In c, one sees a spore strangled by the medium, as if it divided itself transversally. In f, a median disk separates the two polar vacuoles. In h, the chromatin is deposited right against the wall of the spore, eccentrically.

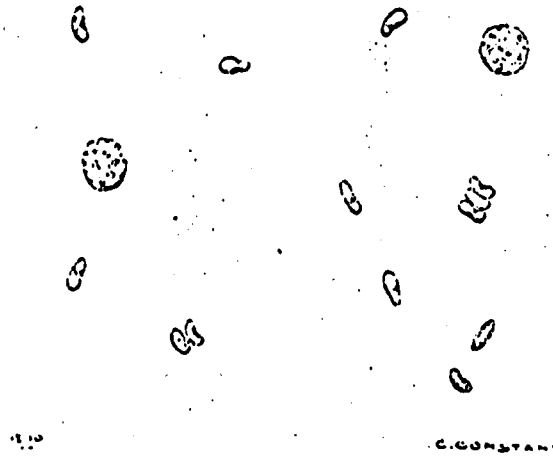


Fig. 7. — Smear preparation of parasited mouse brain. Encephalitozoon isolated or disposed in mass. Coloration with eosin-orange-blue of Unna.

(1) GUYENOT and NAVILLE. Swiss Review of Zoology, 30, no. 1, 1922.

Figure 9 shows spores colored by the safranine. In a, the two nuclei seem to be tied together by a thin filament of chromatin; in b, the two nuclear formations are polar.

2. __ SECTION METHODS. __ Technique: Fixation of the tissues by the Bouin-Brasil liquid, paraffin sections. Methods employed: Mann; iron-hematoxylin; Safranine-picro-indigo-carmin; Twort; Giemsa prolonged (forty-eight hours); Blue polychrome of Unna. This last method comprises a few details.

a) Coloration to orange G (1 p. 100), during twenty minutes; water bath;

b) Coloration to eosin (1 p. 100), thirty to sixty minutes; water bath;

c) Blue polychrome of Unna (pure), twenty minutes; same differenciation and mounting as for the smear preparation.

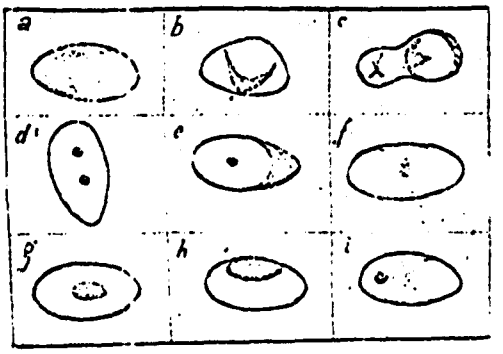


Fig. 8. __ Encephalitozoon cuniculi spores. Smear preparation treated first of all with chlorhydric acid, next colored with iron hematoxylin.

We will study the morphology of the Encephalitozoon cuniculi first of all in the rabbit, then in the rat and the mouse.

a) RABBIT. __ THE parasite could not be distinguished, up until now, except in the encephal and in the kidney.

ENCEPHAL (Brain, central and mesocephal nuclei). __ The parasite exists in variable quantity in the encephal, either at the level of the cortical nucleus and the under-cortical, or, more rarely, far from these nuclei (see Fig. 10). In this last case, one declares it in the interior of the cysts of variable dimension, being able to attain sometimes the dimension of a big pyramidal cell (25 to 30 microns). These cysts, constituted by a thin membrane, are spherical or ovoid; one or two flattened nuclei are molded on their wall. The cyst can enclose an incalculable number of parasites doubled one on top of the other. The spore is oval, bacillary or needle-shaped (cf. Plate III, fig. 6, 7 and 8; Plate IV, fig. 9; in the text, fig. 11).

Fig. 9. __ Same spores, same coloration (cf. fig. 8).

The specialized foveola is replaced by a cyclic colloidal [illegible], or bright ring of cells of the same width either at the center of the yellow spot, or at the edge of the [illegible]. The fovea has the highest visual acuity, or power of discrimination, but a somewhat less color sensitivity than the remainder of the yellow spot. This difference is due chiefly to the yellow pigment being more concentrated at the center of the yellow spot, and partly to the greater density of the cones in the fovea than elsewhere in the yellow spot. The [illegible] is entirely free from blood vessels, and is that of the [illegible]. The [illegible] is nourished by blood from the choroid. It is not reflected by the optic disk from the eye.

On the basal surface, and in nearly [illegible] neighborhood, the [illegible] and [illegible] are located. These are [illegible] in the [illegible] eyeball. However, the [illegible] have a relative magnification, which makes for the fact that they can be distinguished by [illegible].

[illegible] (see Fig. 11). [illegible]

the leucocytic reaction which does not only proceed itself around the cysts, after their breaking out, the parasite is englobed by the macrophages. It degenerates in the protoplasm of these mononuclears, becomes polymorphous, transforms itself into grains colorable by the basic colors and finishes by disappearing completely. Just the same, we met up with quite a few of them conserved in the center of a necrotic foyer occupying the middle of a nodule (coloration by hem). For us, the nodule represents a defensive reaction around the cyst, which, after having broken out, puts the parasites in liberty (cf. Pl. to III, fig. 3 and 4).

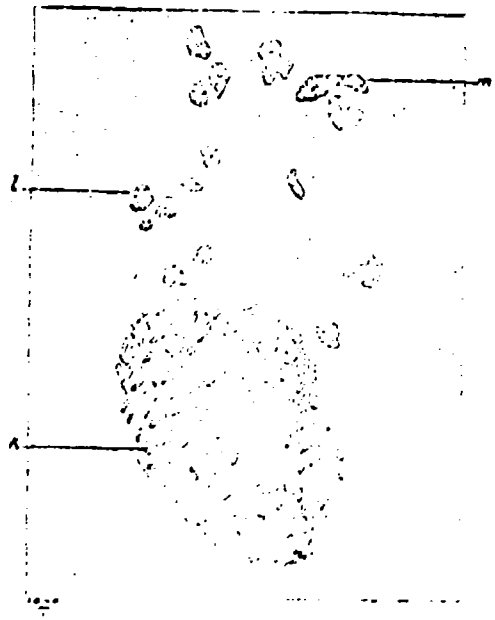


Fig. 11. — Rabbit 33/V, inoculated July 27, 1944, [illegible] with one [illegible] solution from rabbit 30/V, sacrificed [illegible] Granulocytic reaction around the cyst. L, lymphocyte; P, polymorphonuclear. Hem. method.

[illegible]. — The alterations [illegible] the thickened wall of the [illegible]; [illegible] were ruled by [illegible] of the nodular substance and [illegible] connective tissue of the [illegible] degenerative and inflammatory. In the cortical zone, the [illegible] the aspect of a tuberculous cutaneous bone (cf. fig. 14). The dilated tubes are obstructed by an interstitial inflammatory tissue, constituted by lymphocytes, big mononucleated, and, as usual, by a characteristic distribution of necrosed elements.

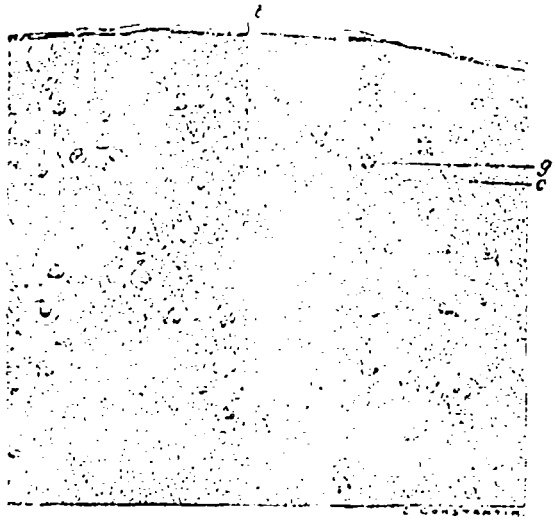


Fig. 14. — [illegible] bone (see fig. 13). — [illegible]. — 1, cortex; 2, [illegible]; 3, [illegible]; 4, [illegible].

In the [illegible] of the papilla, [illegible] a tube (pyelitis tube), in all [illegible] to the [illegible] that are observed in the preceding. In [illegible] of these [illegible] the [illegible] tubules are, either totally or [illegible], as [illegible]. In the last case, there is [illegible] [illegible], constituted by a pile of epithelial cells [illegible] and of [illegible] polynuclear globules, in a state of [illegible]. Cocci are [illegible] these nodules.

[illegible] can there be discovered easily, above all when one uses [illegible], or still the [illegible] of Unna procedure (2).

(2. [illegible] emulsion [illegible], I think to using Curtis's procedure (orcein-[illegible]-carmin).

It multiplies rapidly exclusively in the epithelial cells which cover the renal tubes. One there vesicular cysts of variable size, containing 3 to 4 germs, or several groups of ten of spores (cf. fig. 19). In the

FIG. 18. — [illegible], on rabbit November 22, 1943, with [illegible]; [illegible]; intra-cerebral [illegible]; [illegible]: E, renal tubes, [illegible]. All of the [illegible] cells (E, Ir) are parasited. [illegible], in which [illegible] and the contains were spread in the [illegible]. [illegible].

beginning, the parasited epithelial cells are absolutely normal; the protoplasm colors well; the nucleus, well developed near the periphery offers a reticulum of chromatin and a nucleole in perfect state. Later on, the cyst, while being beyond measure, bursts and the spores spread in the light

of the e ncoules to be rejected (tuble with the urinary secretion (cf. [illegible] III, fig. 5, 7 and 8).

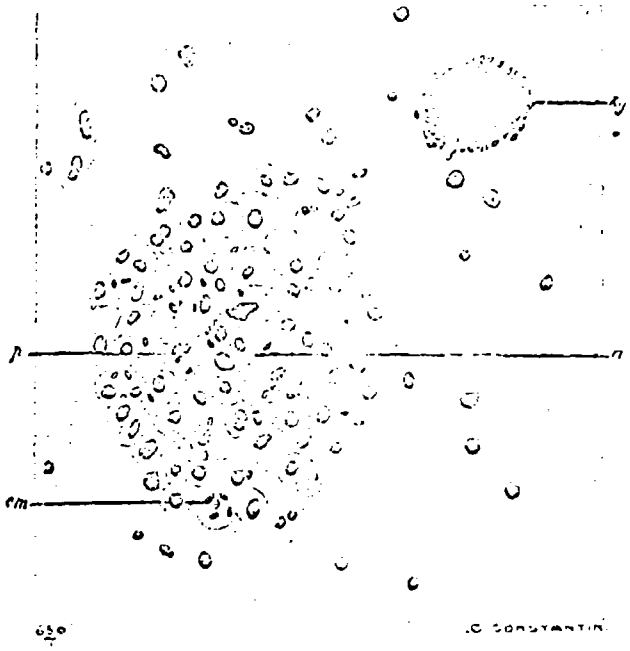


fig. [illegible]. [illegible] fig. [illegible]

[illegible]

The [illegible] are to be put in evidence in the center of the nodule, [illegible] the intralobular debris and leucocytic debris which constitute [illegible] described here above. Nevertheless, their aspect seems to [illegible]. The cellular inventory [illegible], beer's drinkers, [illegible], which entered in contact with urinary [illegible]; in brief, they offer [illegible] what one does find in the inter-canalicular granulations.

[illegible] the beginning the [illegible], the other nodules showed no [illegible]. In one [illegible] the [illegible] within the [illegible], the [illegible], [illegible], the [illegible] with the [illegible], the [illegible], the [illegible], the [illegible], the [illegible], the [illegible], the [illegible], the [illegible], etc... [illegible] in which [illegible], the [illegible] [illegible] the [illegible]. Thus, however, with the case of those who [illegible], as on the liver, the [illegible] the lung.

The spleen sometimes presents a myeloid transformation of the most acute order, with myelocytes and [illegible]. The splenic sinuses close in great [illegible] of blood or ocher pigment. In the liver and the lung, one distin[illegible] myelocytic nodules; it is the same thing in the [illegible]. But, still again, all of these lesions are devoid of parasites.

b) MOUSE. — The mouse, like the rabbit, can present not only intracerebral cysts, but also perivascular disks and genuine nodules. These last mentioned are constituted by a pile of cells of an epithelioid aspect, with abundant protoplasm, colorable in greenish blue by Lehmann. (cf. Fig. 14, 15 and 16). These cells enclose typical spores and have the aspect of

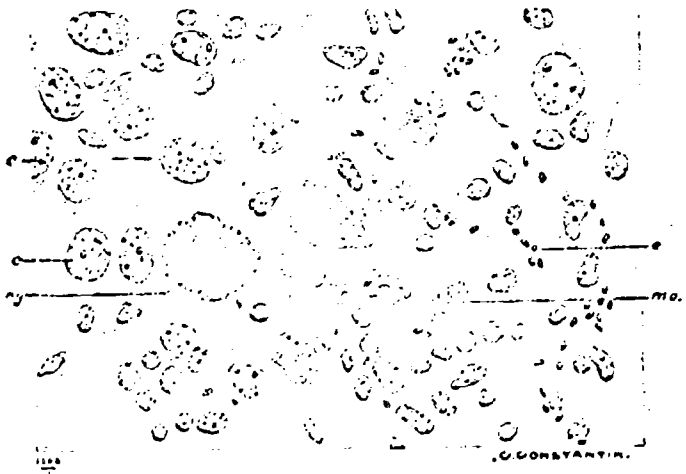


Fig. 15. — Lesions of [illegible] mouse, spontaneously [illegible]. — [illegible], [illegible] corn; 2, nerve cells of normal [illegible]; 1, cyst clinging in numerous [illegible] spores; inflammatory [illegible] in the [illegible] corn, with 1, mononuclear cells, and 2, free or [illegible] spores. Giemsa method.

[illegible] with [illegible] cells, multiple lesions were able to be observed in the cerebellum cranial nerve. In several animals, we verified, at the level of posterior cornua, not only perivascular discs, but still quite voluminous parasitic cysts. Finally, in a single mouse, [illegible] examined, we found cysts in the thymus, while 91 of these mice showed parasites in the [illegible].

- We must mention briefly the [illegible], multinucleated, of [illegible] in the tissue of the [illegible] of the rat (cf. Fig. 2 of [illegible]). In the mouse, the [illegible] in the protoplasm of the [illegible] cells; in the rat, it was a question of hemorrhagic nodules with a [illegible] center, clearly [illegible], although lightly deformed.

* * *

In sum, the study of the morphological [illegible] of Trypanosoma cruzi, on one hand, [illegible] with the [illegible] of [illegible]

[illegible]

[illegible]

[illegible]

[illegible]

(1) Cysts of [illegible] [illegible] [illegible]

[illegible]

Concerning [illegible] of cells [illegible] (This [illegible] [illegible]).

[illegible]

CHAPTER IV

EVOLUTION OF THE MACROGAMETOCYTE

The researches, still much [illegible], which I have continued, and for some having precised the diverse successive phases of the [illegible] gametocyte. Studies on the Microsporidies (1), and in particular that which Caullery and Mesnil (loc. cit.) entrusted to the Microsporidia of the eruche (Plodia interpunctella), show that the evolutive cycle of these organisms is very complex. However, we succeeded in getting in evidence [illegible] phases of this cycle, in particular the [illegible], that [illegible] as well as in the mouse.

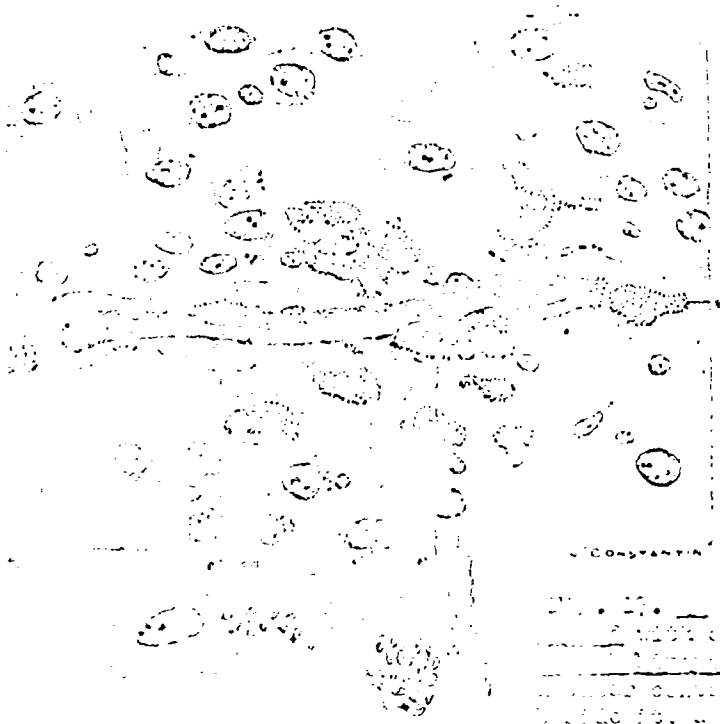
1 - IN THE MOUSE. - An oval hyaline cytoplasm, poor in [illegible], was inoculated in the peritoneum of several mice. One or two days after the inoculation. The [illegible] contains a number of parasites included in a hyaline vacuole. [illegible] pseudopodial cells is projected to the periphery, the protoplasm [illegible] vacuole filled with a mass. The [illegible] is present [illegible] of the [illegible] cell, included in Leydig's cells.

In addition, in the same animal a typical (sporogonous infection), [illegible] encysted contains numerous smaller cysts than the habitual cysts, [illegible] with young sporozoites (sporoblasts), limited by a chitinous envelope and an [illegible] protoplasmic mass (sporoplasm - merozoites).

2 - IN THE RAT inoculated by peritoneal route, we disclosed, on smear preparations of peritoneum, voluminous hyaline cells, containing pigment granules spread (Fig. 17); [illegible] cells (Fig. 17, at the bottom and to the left) offer a hyaline projection towards the periphery and a [illegible] containing a chromatin of formation. These are constituted by two granulations of chromatin, appearing tied by a filament, they are surrounded by a capsule.

In these cells, the multiplication of these merozoites of contaminated mouse is [illegible] the procedure, [illegible] only of spores arrived at the ultimate stage of their development, such as is described that in the course of this [illegible], but still young spores (sporoblasts), these distinct from

(1) BALBIANI. Leçons sur les Sporozoaires, Paris, Tome, 1884.


Constantin

[illegible]

[illegible]

[illegible]

[illegible]

---

(1) [illegible]
(2) [illegible]
(3) [illegible]

[illegible]

[illegible] (2).

[illegible] 1[illegible], 19[illegible].

Protocol [illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	S. [illegible] jours	M. [illegible] jours	S. [illegible]
Coupes [illegible]	Posit.	Posit.	Posit.
[illegible]	Posit.	0	Posit.

[illegible]

[illegible]

(1) [illegible]

Protocole II. — *Virus Tissot.*

Virus cérébral du lapin 100, inoculé dans le cerveau des lapins : [illegible] au lapin blanc.

N° du lapin		149	150	151	153	152	145
Mort ou sacrifié.		M. 83 j.	M. 65 j.	S. 102 j.	S. 102 j.	S. 102 j.	S. 102 j.
Frottis	Cerv.	*Posit.*	0	0	0	*Posit.*	0
	Rein.	0	0	*Posit.*	*Posit.*	0	0
Coupes cerv.	Lés.	0	0	*Posit.*	*Posit.*	0	0
	Par.	0	0	0	0	0	0
Coupes rein.	Lés.	0	0	*Posit.*	*Posit.*	*Posit.*	*Posit.*
	Par.	0	0	*Posit.*	*Posit.*	0	*Posit.*

Protocole III. — *Virus Thalhimer.*

Virus humain 660-4-2, passé deux fois sur lapin, inoculé dans le cerveau des lapins :

[illegible] 660-4-2, passé [illegible] sur lapin, inoculé[illegible] au rabbit brains.

Mort ou sacrifié.			S. 157 jours	S. 157 jours
Numéro des lapins.			56 V	56 V
Coupes cerv.	Lés.		*Posit.*	0
	Par.		*Posit.*	0
Coupes rein.	Lés.		*	*Posit.*

Protocole IV.

Virus cérébral 56/V (Pasteur), inoculé dans le cerveau des lapins : [illegible] au lapin blanc.

Numéro des lapins		31 V	32 V	34 V	35 V	36 V
Mort ou sacrifié.		S. 83 j.	M. 83 j.	S. 106 j.	S. 106 j.	S. 116 j.
Frottis	Cerv.	*	*	*	*	*
	Rein.	*	*	*	*	*
Coupes cerv.	Lés.	0	*Posit.*	*Posit.*	*Posit.*	*Posit.*
	Par.	0	0	*Posit.*	*Posit.*	*Posit.*
Coupes rein.	Lés.	*	*	*Posit.*	*Posit.*	*Posit.*
	Par.	*	*	*Posit.*	*Posit.*	0

Numéro des lapins		36 C	46 V	52 V	57 V	59 V
Mort ou sacrifié.		S. 116 j.	S. 148 j.	S. 148 j.	S. 153 j.	S. 148 j.
Frottis	Cerv.	*	*	*Posit.*	*	0
	Rein.	*	*	*Posit.*	*	0
Coupes cerv.	Lés.	*Posit.*	*Posit.*	*Posit.*	*Posit.*	0
	Par.	*Posit.*	*Posit.*	*Posit.*	*Posit.*	0
Coupes rein.	Lés.	*Posit.*	*	*Posit.*	*Posit.*	*Posit.*
	Par.	*	*	*Posit.*	*Posit.*	0

Protocole V.

Virus cérébral des lapins 39/V, 40/V et 41/V, inoculé dans le péritoine et le cerveau des lapins :

		DANS LE PÉRITOINE			DANS LE CERVEAU ET LE PÉRITOINE	
Numéro des lapins		1/B	2/B	3/B	4/B	
Mort ou sacrifié		S. 147 j.	M. 75 j.	S. 147 j.	M. 125 j.	M. 80 j.
Frottis	Cerv.	*Posit.*	0	*Posit.*	0	0
	Rein.	0	*Posit.*	*Posit.*	0	0
Coupes cerv.	Lés.	*Posit.*	0	*Posit.*	0	0
	Par.	0	0	*Posit.*	0	0
Coupes rein.	Lés.	0	*Posit.*	*Posit.*	0	0
	Par.	0	*Posit.*	*Posit.*	0	0

Protocole VI.

Virus cérébral des lapins 36/V, 37/V et 40/V, inoculé dans le cerveau des lapins :

		41/B	74/B	76/B	77/B
Numéro des lapins					
Mort ou sacrifié		M. 76 j.	M. 82 j.	M. 100 j.	S. 120 j.
Frottis	Cerv.	0	0	0	*Posit.*
	Rein.	*Posit.*	0	*Posit.*	0
Coupes cerv.	Lés.	0	0	*Posit.*	0
	Par.	0	0	*Posit.*	0
Coupes rein.	Lés.	0	0	*Posit.*	*Posit.*
	Par.	0	0	*Posit.*	0

Protocole VII.

Virus cérébral des lapins 31/V et 32/V, inoculé dans le cerveau des lapins :

Numéro des lapins		8	72	77	94	95	99
Mort ou sacrifié		M. 110 j.	S. 110 j.	S. 113 j.	S. 123 j.	M. 124 j.	S. 125 j.
Frottis	Cerv.	0	0	0	0	0	0
	Rein.	0	0	0	0	*Posit.*	0
Coupes cerv.	Lés.	*Légère*	0	0	0	0	0
	Par.	0	0	0	0	0	0
Coupes rein.	Lés.	0	0	0	0	*Posit.*	0
	Par.	0	0	0	0	*Posit.*	0

Protocole VIII.

Virus rénal des lapins 36/V, 70/V et 40/V, inoculé dans le cerveau des lapins :

Numéro des lapins		71 B	76 B	87 B	91 B
Mort ou sacrifié		M. 71 j.	M. 81 j.	S. 133 j.	M. 107 j.
Frottis	Cerv.	*Posit.*	*Posit.*	0	0
	Rein	*Posit.*	0	*Posit.*	0
Coupes cerv.	Lés.	0	0	*Légères.*	0
	Par.	0	0	0	0
Coupes rein.	Lés.	0	0	*Posit.*	0
	Par.	0	0	*Posit.*	0

Protocole IX.

Virus rénal du lapin 37/V, inoculé dans le cerveau des lapins :

Numéro des lapins		93	97
Mort ou sacrifié		M. 75 jours	S. 112 jours
Frottis	Cerv.	0	0
	Rein	0	0
Coupes cerv.	Lés.	0	*Légères.*
	Par.	0	0
Coupes rein.	Lés.	0	0
	Par.	*Posit.*	0

Protocole X.

Virus rénal des lapins 33/V et 37/V, inoculé dans le cerveau des lapins :

Numéro des lapins		83	86
Mort ou sacrifié		M. 85 jours	S. 125 jours
Frottis	Cerv.	0	*Posit.*
	Rein	0	0
Coupes cerv.	Lés.	0	*Posit.*
	Par.	0	*Posit.*
Coupes rein.	Lés.	0	0
	Par.	0	0

Protocole XI.

Virus rénal du lapin 42/V, inoculé dans le cerveau des lapins :

Numéro des lapins		305	308
Mort ou sacrifié		M. 61 jours	M. 95 jours
Frottis	Cerv.	0	0
	Rein	0	*Posit.*
Coupes cerv.	Lés.	0	*Posit.*
	Par.	0	0
Coupes rein.	Lés.	0	*Posit.*
	Par.	0	*Posit.*

Protocole XI *bis*.

Virus rénal du lapin 42 V, inoculé dans le nerf sciatique des lapins :

Numéro des lapins		37/A	38/A
Mort ou sacrifié		M. 4 jours	S. 116 jours
Frottis	Cerv.	*Posit.*	0
	Rein.	*Posit.*	0
Coupes cerv.	Lés.	*Posit.*	0
	Par.	*Posit.*	0
Coupes rein.	Lés.	*Posit.*	0
	Par.	*Posit.*	0
Nerf sciatique		0	

Protocole XII

Virus rénal du lapin 42 V, inoculé dans les reins des lapins :

Numéro des lapins		41/A	42/A	43/A	40/A
Mort ou sacrifié		M. 3 j.	S. 111 j.	S. 111 j.	S. 111 j.
Frottis	Cerv.	0	*Posit.*	*Posit.*	*Posit.*
	Rein.	*Posit.*	*Posit.*	0	0
Coupes cerv.	Lés.	0	*Posit.*	*Posit.*	*Posit.*
	Par.	0	*Posit.*	*Posit.*	0
Coupes rein.	Lés.	0	*Posit.*	*Posit.*	*Posit.*
	Par.	*Posit.*	0	0	0

Protocole XIII.

Virus cérébral et rénal du lapin 71 B, inoculé dans les testicules des lapins :

Numéro des lapins		1/A	2/A
Mort ou sacrifié		M. 32 jours	M. 32 jours
Frottis	Cerv.	0	0
	Rein.	*Posit.*	*Posit.*
Coupes cerv.	Lés.	0	0
	Par.	0	0
Coupes rein.	Lés.	0	*Posit.*
	Par.	0	*Posit.*

Protocole XIV.

Liquide péritonéal du lapin 4/IV, inoculé dans le cerveau du lapin :

Numéro du lapin		65/B
Mort ou sacrifié		S. 132 jours
Frottis	Cerv.	0
	Rein.	0
Coupes cerv.	Lés.	0
	Par.	0
Coupes rein.	Lés.	0
	Par.	0

Protocole XV.

Infection par cohabitation avec les lapins infectés du protocole IV. Lapins sains :

Numéro des lapins		41/V	42/V
Mort ou sacrifié		S. 147 jours	S. 105 jours
Coupes cerv.	Lés.	0	*Posit.*
	Par.	0	*Posit.*
Coupes rein.	Lés.	»	*Posit.*
	Par.	»	*Posit.*

Protocole XVI.

Infection par cohabitation, avec les lapins infectés du protocole IV. Lapins sains :

Numéro des lapins		50	51
Mort ou sacrifié		M. 60 jours	S. 119 jours
Frottis	Cerv.	0	*Posit.*
	Rein.	0	*Posit.*
Coupes cerv.	Lés.	0	*Posit.*
	Par.	0	*Posit.*
Coupes rein.	Lés.	0	*Posit.*
	Par.	0	*Posit.*

Protocole XVII.

Urine des lapins 33/V et 37/V, inoculée dans le cerveau du lapin :

Numéro du lapin.		91
Mort ou sacrifié		S. 125 jours
Frottis	Cerv.	0
	Rein.	0
Coupes cerv.	Lés.	*Posit.*
	Par.	*Posit.*
Coupes rein.	Lés.	*Posit.*
	Par.	*Posit.*

Protocole XVIII.

Urine du lapin 42/V, administrée « *per os* » :

Numéro du lapin.		100
Mort ou sacrifié		S. 105 jours
Frottis	Cerv.	0
	Rein.	*Posit.*
Coupes cerv.	Lés.	*Posit.*
	Par.	*Posit.*
Coupes rein.	Lés.	*Posit.*
	Par.	*Posit.*

Protocole XIX.

Emulsion cérébrale fraîche des lapins 66/V, 76/U et 66/V, inoculée dans le cerveau des lapins :

Numéro des lapins		72 B	73 B	93 B	95 B
Mort ou sacrifié		S. 84 j.	S. 125 j.	S. 84 j.	S. 125 j.
Frottis	Cerv.	0	0	0	0
	Rein.	0	0	0	0
Coupes cerv.	Lés.	0	0	0	0
	Par.	0	0	0	0
Coupes rein.	Lés.	0	0	0	0
	Par.	0	0	0	0

Protocole XX.

Emulsion cérébrale filtrée des lapins 53/V et 57/V, inoculée dans le cerveau des lapins :

Numéro des lapins		71	79	93	91
Mort ou sacrifié		S. 111 j.	S. 111 j.	S. 111 j.	M. 56 j.
Frottis	Cerv.	0	0	0	0
	Rein.	0	0	0	0
Coupes cerv.	Lés.	0	0	0	0
	Par.	0	0	0	0
Coupes rein.	Lés.	0	0	0	0
	Par.	0	0	0	0

Numéro des lapins		92	70	377
Mort ou sacrifié		S. 111 j.	S. 102 j.	S. 111 j.
Frottis	Cerv.	0	0	0
	Rein.	0	0	0
Coupes cerv.	Lés.	0	0	0
	Par.	0	0	0
Coupes rein.	Lés.	0	0	0
	Par.	0	0	0

Protocole XXI.

Emulsion cérébrale du lapin 42/V, inoculée dans le cerveau des *cobayes* :

Numéro des cobayes		21 A	22/A
Mort ou sacrifié		M. 28 jours	M. 45 jours
Frottis	Cerv.	0	0
	Rein.	0	0
Coupes cerv.	Lés.	0	0
	Par.	0	0
Coupes rein.	Lés.	0	0
	Par.	0	0

Protocole XXII.

Emulsion cérébrale du lapin 42/V, inoculée dans le péritoine des *cobayes* :

	48/A	49/A
Numéro des cobayes		
Mort ou sacrifié	M. 41 jours	S. 105 jours
Frottis Cerv.	0	0
Frottis Rein	0	*Posit.* (rares spores).
Coupes cerv. Lés.	0	0
Coupes cerv. Par.	0	0
Coupes rein. Lés.	0	0
Coupes rein. Par.	0	0

Protocole XXIII.

Emulsion rénale du lapin 42/V, inoculée dans le péritoine des cobayes :

	50/A	52/A
Numéro des cobayes		
Mort ou sacrifié	M. 41 jours	S. 105 jours
Frottis Cerv.	0	0
Frottis Rein.	0	0
Coupes cerv. Lés.	0	0
Coupes cerv. Par.	0	0
Coupes rein. Lés.	0	0
Coupes rein. Par.	0	0

Protocole XXIV.

Emulsion cérébrale souris 4 (série V), inoculée dans le cerveau du *chien* :

	2
Numéro du chien	M. 25 jours
Mort ou sacrifié	*Posit.*
Frottis Cerv	0
Frottis Rein	0
Coupes cerv. Lés.	0
Coupes cerv. Par.	0

Protocole XXV.

Emulsion cérébrale souris 4 (série V), inoculée dans le cerveau du *singe* :

	Mac. cynomolgus.
Singe	S. 32 jours
Mort ou sacrifié	0
Frottis cerv	0
Coupes cerv. Lés.	0
Coupes cerv. Par.	0

Protocole XXVI.

Emulsion cérébrale du lapin n° V, inoculée dans le péritoine des rats :

		R/1	R/2	R/3	R/4
Numéro des rats					
Mort ou sacrifié		M. 22 j.	M. 60 j.	M. 60 j.	M. 67 j.
Poids	Testicule	*Perd.*	"	"	"
	Cerv.	"	*Perdt.*	0	0
Coupes cerv.	Lues	0	0	0	0
	Par.	0	0	0	0

Protocole XXVII.

Emulsion cérébrale du lapin 42 V, inoculée dans le péritoine des rats :

		R/1	R/2	R/3	R/4
Numéro des rats					
Mort ou sacrifié		M. 21 j.	M. 42 j.	M. 42 j.	M. 47 j.
Poids	Cerv.		*Perdt.*	0	0
Coupes cerv.	Lues	0	0	0	0
	Par.	0	0	0	0
Coupes foie.	Lues	"	"	"	"
	Par.	*Perdt.*	"	"	"

Protocole XXVIII.

Transmission héréditaire de l'[illegible] chez la souris :

Petite née de mère n° 1 [illegible] cerveau lucre = 0.

Série B : 5 petits de 8 jours. Tous négatifs.
Série C : 2 petits de 26 jours. Tous négatifs.
Total : 7 petits, 7 négatifs.

[illegible] de souris baptisées

Série A : 7 petits âgés de [illegible] Tous 0.
— A' : 6 petits — de [illegible]
— A'' : 15 petits — de [illegible]
— B : 6 petits — de [illegible]
— D : 6 petits — [illegible] négatifs et 1 *positif*.
— 7 : 1 petit âgé de [illegible]
— 7 : 1 petit — de [illegible]
— 1 : 1 petit — de [illegible]
— 10 : 1 petit — de 10 jours. *Négatif*.
Total : 57 petits âgés de 1 heures à 30 jours. 56 négatifs et 1 *positif*.

[illegible].

[illegible]

[illegible]

[illegible]